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(54) Title: HIV REVERSE TRANSCRIPTASE VACCINE

(57) Abstract

A synthetic vaccine comprises a recombinant vaccine vector that incorporates a nucleotide sequence capable of being expressed as all or part of an HIV reverse transcriptase. The vector is combined with all or part of a purified HIV reverse transcriptase in an adjuvant. The vaccine vectors include poxvirus, herpes virus, and adenovirus. The synthetic vaccine applies particularly to the augmentation of immune responses in HIV-infected asymptomatic, immunosuppressed, or immunodeficient animals or humans.

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HIV REVERSE TRANSCRIPTASE VACCINE

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention relates to synthetic vaccines for immunizing against human immunodeficiency virus (HIV). More particularly, the invention relates to recombinant vaccine vector-encoded HIV reverse transcriptase (RT), combined with at least one booster inoculation of purified HIV RT in an adjuvant.

2. Related Art

HIV, a human retrovirus, causes both acquired immune deficiency syndrome (AIDS) and acquired immune deficiency related complex (ARC). During the past decade, this virus has infected 5 to 10 million people worldwide and caused over 200,000 deaths due to complications arising from AIDS. Many attempts have been made to immunize animals and humans with prototype vaccines, but none of them have been particularly effective in protecting against or moderating the onset of the disease.

Development of an effective AIDS vaccine has been hampered by severe problems. Most present research strategy produces vaccines based on the glycoprotein envelope of HIV. However, the amino acid sequence of the glycoprotein envelope, gp120, exhibits a high degree of variability among virus particles obtained from different patients, or even from the same patient at different times. These ongoing mutations in the virus envelope lead to ineffective vaccines that fail to provide an immune response to the constantly changing HIV.

Certain criteria are necessary for an effective vaccine against HIV: (1) an HIV portion that elicits the most powerful natural immune response; (2) an HIV portion whose amino acid sequence is reasonably conserved; (3) protection against any HIV isolate; (4) a high enough level of immune response amplification to overcome HIV; (5) an immunogen that produces humoral and cell-mediated immunity; and (6) an immunogen that excludes sites which might induce deleterious side effects.

Therefore, a need continues to exist for a vaccine that provides both protective immunity to HIV-negative individuals and effective therapy to HIV-positive asymptomatic individuals. The present invention provides just such a vaccine using HIV RT to induce both a humoral and a cytotoxic T-cell response.

SUMMARY OF THE INVENTION

In one aspect, this invention provides a synthetic vaccine. It comprises a recombinant vaccine vector that incorporates a nucleotide sequence capable of being expressed as all or part of an HIV RT. The vector is combined with all or part of a purified HIV RT in an adjuvant. Both the HIV RT expressed by the recombinant vector and the purified HIV RT in the adjuvant can be a 66 kDa subunit of the HIV RT.

In another aspect, the present invention provides a method for producing an immune response in an animal or human by the step of administering a synthetic vaccine, as broadly described above.

The present invention applies particularly to the augmentation of immune responses in HIV-infected asymptomatic, immunosuppressed, or immunodeficient animals or humans. In one important aspect, a synthetic vaccine is used in the treatment of HIV-infected asymptomatic, immunosuppressed, or immunodeficient animals or humans. The synthetic vaccine is made up of two parts: (1) a recombinant vaccine vector that incorporates a nucleotide sequence capable of being expressed as all or part of an HIV RT, and (2) at least one booster inoculation of purified HIV RT in adjuvant to enhance the immune response of the animal or human.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1 schematically represents the construction of the vaccinia recombination vector;

FIG. 2 shows a Western blot of VV:RT-infected cell lysates;

FIGS. 3a and 3b are Western blots showing a time course of expression study to detect HIV RT;

FIG.4 shows an antibody response in VV:RT-infected mice and rabbits;

FIG. 5 shows the longitudinal titers of anti-RT antibodies in rabbits;

FIG. 6 shows antibody response against HIV RT in African green monkeys;

FIG. 7 is a graph showing the results of an assay to determine the titer of the anti-RT antibody in African green monkey sera; and

FIG. 8 is a graph showing the results of an assay to determine the titer of RTI antibodies in vaccinated African green monkeys.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a synthetic vaccine containing HIV RT, which acts as an immunotherapeutic or immunopreventive agent for the treatment of HIV infected individuals. The approach is to produce a synthetic vaccine that consists of two parts. The first part is in the form of a recombinant vaccine vector, such as a recombinant vaccinia virus (rVV). The rVV was constructed by inserting a nucleotide sequence from the HIV viral genome into a wild-type vaccinia virus (VV). That portion of the HIV genome thus becomes expressed by the recombinant virus. Specifically, the invention inserts a nucleotide sequence that encodes the HIV RT gene into VV, so that the rVV functions as a vaccine when inoculated into humans. Although this invention is primarily described with reference to VV as the vaccine vector, other vaccine vectors (such as other poxviruses, herpes virus, adenovirus, or bacteria) may be used. The second part of the vaccine, consisting of purified HIV RT in adjuvant, provides a booster effect.

HIV RT is a suitable target for immunopreventative measures against HIV, as shown by the following: (1) a correlation between progression to acute AIDS and the loss of antibodies against HIV RT (Laurence, et al., *Science*, 235:1501-1504, 1987), (2) the presence of cytotoxic T lymphocytes directed against HIV RT in infected individuals (Walker, et al., *Science*, 240:64-66, 1988), and (3) the expression of the HIV RT antigen on CD4-positive cells in the peripheral circulation of HIV-seropositive individuals. See U.S. Patent No. 4,904,581.

By immunizing HIV-infected asymptomatic, immunosuppressed, or immunodeficient individuals and periodically boosting with pure HIV RT, the viral spread and progression to AIDS could be effectively controlled. It is also conceivable that, even if an anti-RT vaccination of HIV-negative individuals does not protect them from HIV infection, it could, for the same reasons, at least control the disease. The use of VV as a live vaccine vector for the HIV RT gene (1) facilitates a humoral and cellular immune response to HIV RT and (2) provides prophylactic protection against HIV infection or assists in therapy.

The current knowledge of the genetic structure of HIV includes ten genes that are bounded by long terminal repeats (LTRs). LTRs are sequences at both ends of the HIV DNA that do not code for any protein. Instead, they initiate the expression of viral proteins. Only three of the HIV genes, *gag*, *pol*, and *env*, encode components of virus particles. The other HIV genes regulate the expression of these three genes. The amino acid homology between at least 14 different HIV isolates has shown the *pol* gene of HIV to be the most highly conserved (Myers, et al., eds., *Human Retroviruses and AIDS*, 1990, Los Alamos National Laboratory). The amino acid homology in the *pol* gene was 81% between isolates, compared with the *gag* gene, which was 71%, and the *env* gene, which was 44%. When the region encoding the RT polypeptide in *pol* is compared, an 83% homology is observed. The conserved nature of RT supports its use as a vaccine, because, unlike the envelope gene, the degree of antigenic drift is minimal.

Previous efforts have focused on expression of the entire *pol* open reading frame in rVV infected cells. This strategy required proteolytic processing of the *pol* polyprotein into protease, HIV RT, and integrase in order to obtain native HIV RT (Farmerie, et al., *Science*, 236:305-308,1987). The native HIV RT underwent further proteolytic processing by the HIV protease. The HIV was partially cut an additional time, a small sequence at the C-terminus removed, and a heterodimer, containing 66 and 51 kDa subunits, formed that had common N termini. This further cleavage of the HIV RT may affect the characteristics of the enzyme. In addition, this essential proteolysis is inefficient and results in low levels of native HIV RT in rVV infected cells. Animals infected with these initial rVV constructs developed low or undetectable immune responses to *pol* gene products.

To eliminate the need for proteolysis leading to the heterodimer, a different set of rVV's were constructed, using only the coding sequences for native HIV RT. This was accomplished using specially designed oligonucleotide primers and the polymerase chain reaction (PCR). The method allowed isolation of a segment of the *pol* region that encoded only a 66 kDa subunit of those amino acids comprising the native HIV RT. The resulting rVV generated high levels of HIV RT in infected cells and significant antibody titers in vaccinated mice, rabbits, and monkeys.

HIV-infected individuals have been shown to present RT antigens on T-cells at early stages of HIV infection. A monoclonal antibody against HIV RT specifically binds to CD4-positive cells in the peripheral circulation of HIV-seropositive individuals. See U.S. Patent No. 4,904,581.

If these RT-presenting cells represent the HIV-infected cell population, a strong cytotoxic T-cell response against HIV RT could protect an individual by clearing infected cells before the virus has a chance to spread.

HIV-infected individuals are known to develop antibodies against HIV RT without this enzyme being presented to the immune system by viral particles. HIV is unique among retroviruses, including HTLV-I and HTLV-II, in its ability to induce anti-RT antibodies. Next to the envelope protein gp41, the most frequently recognized viral antigen in seropositive individuals are the p51, p66 proteins which comprise RT. Furthermore, a correlation has been shown between the loss of RT-inhibiting (RTI) antibody and worsening clinical disease (Laurence, et al., *Science*, 235:1501-1504, 1987; Advani, et al., *J.Clinical Microbiology*, 27:1453-1455, 1989). The loss of antibodies capable of inhibiting HIV RT activity occurs, even though RT-binding antibodies are present (DeVico, et al., *AIDS Res. Hum. Retroviruses*, 4:17-21, 1988). The presence or absence of RTI antibody has been proposed as a marker of advanced AIDS. Therefore, a vaccine that induces RTI antibodies in an individual, with the use of a boost regimen that keeps RTI antibody levels high, could prevent the progression of AIDS.

Because T-cell-mediated cytotoxicity is an important factor in the control of infections including HIV, the presence of RT-specific cytotoxic T-lymphocytes (CTL) is an important observation in infected individuals. Several groups have characterized the presence of RT-specific CTLs in HIV-infected humans, and recent reports have identified RT-specific CTL epitopes (Walker, et al., *Science*, 240:64-

66,1988; Walker, et al., *Proc. Natl. Acad. Sci. USA*, 86:9514-9518, 1989; Hosmalin, et al., *Proc. Natl. Acad. Sci. USA*, 87:2344-2348, 1990).

The observed degree of difficulty in culturing HIV from the peripheral blood mononuclear lymphocytes of seropositive individuals has been correlated with the serum level of RTI antibodies (Sano, et al., *J. Clin. Microbiol.*, 25:2415-2417, 1987). This observation suggests that the presence of high RTI antibody levels may decrease the replication of virus in infected individuals. If that is correct, it supports the argument for continued immunization against RT in infected individuals.

Human subjects responded marginally to primary inoculation with vaccines (VAC/ENV) that consisted of vaccinia recombinant virus expressing the HIV envelope glycoprotein, gp160. However, such subjects demonstrated a significant boost in humoral, as well as cell-mediated immunity, following a boost with purified envelope glycoprotein (Hu, et al., *Abstracts from VI International Conference on AIDS*, Th.A.343, 1990). Individuals with clinical and laboratory evidence of prior smallpox immunization demonstrated a low primary response to VAC/ENV but a subsequent boost effect with purified protein was observed. Additionally, in a murine system the combination of first priming with VAC/ENV, followed by the purified protein boost, resulted in a greater humoral immune response than either of the two regimens by themselves (Cooney, et al., *Abstracts from VI International Conference on AIDS*, Th.A.333, 1990). The

studies of Hu, et al. and Cooney, et al. above show that a successful immunization for HIV is possible by first priming with a vaccinia-HIV construct expressing gp160, and then following with a boost of the purified gp160 in adjuvant.

In an early and promising Phase I vaccine trial, ARC and AIDS patients were immunized against an HIV preparation to prevent worsening clinical status (Picard, et al., *Lancet*, 336:179, 1990). The vaccine consisted of *vac-pol*, *gag*, and *env* constructs, as well as immunogenic synthetic peptides from the same proteins. Autologous cells were isolated from each individual, infected with the vaccinia constructs *in vitro*, fixed with paraformaldehyde, and re-innoculated into the patient. In 19 vaccinated patients no opportunistic infection episodes were observed, and weight was maintained out to 9 months post-vaccination. Additionally, the absolute levels of CD4 cells stabilized or increased significantly in the vaccinated individuals as compared with a control group. These encouraging results indicate that therapeutic vaccination of seropositive individuals might be an effective therapy. Vaccination with the vaccinia construct-infected cells may be an important part of the immunization protocol, because these cells are expressing HIV epitopes in association with class I MHC determinants. This is essential for the induction of CD8 positive cytotoxic cells. Significantly, soluble proteins found in conventional vaccines do not efficiently generate these types of effector cells.

The administration of the rVV is generally by means of scratching the skin and application of infectious rVV.

A localized lesion develops as a result of infection of cells at the site of administration. Non-sterile conditions are often acceptable. For the purified HIV RT, administration is slightly more complex. The HIV RT can be prepared as an injectable, either as a liquid solution or suspension; a solid form suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation may also be emulsified. The HIV RT can be mixed with excipients which are pharmaceutically acceptable and compatible. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like, and combinations thereof. In addition, the HIV RT can be combined with minor amounts of auxiliary substances such as wetting or emulsifying agents, or pH buffering agents.

The invention encompasses the use of the purified HIV RT in adjuvant as a booster inoculation to enhance the immune response. Normally, the adjuvant and the HIV RT are mixed prior to presentation to the immune system, or presented separately, but into the same site of the animal or human being immunized. Adjuvants can be loosely divided into several groups based on their composition. These groups include water-in-oil emulsion adjuvants, such as complete Freund's adjuvant, in which living or dead mycobacteria are suspended. The intense, chronic inflammation around the deposits of emulsion of this adjuvant precludes it use in man. However, incomplete Freund's adjuvant which has an emulsion without mycobacteria is less irritating and has been used clinically. Other groups of adjuvants include mineral salts (for example, $AlK(SO_4)_2$, $AlNa(SO_4)_2$, $AlNH_4(SO_4)$, silica, alum, $Al(OH)_3$, $Ca_3(PO_4)_2$, kaolin, and carbon), polynucleotides (for example, poly IC and poly AU acids), and certain natural substances (for example,

wax D from *Mycobacterium tuberculosis*, as well as substances found in *Corynebacterium parvum*, *Bordetella Pertussis*, and members of the genus *Brucella*). Another substance useful as an adjuvant is Quil A, a crude mixture of saponins that has detergent-like properties.

The purified HIV RT in adjuvant is conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories, intranasal aerosols, and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium carbonate, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations, or powders, and contain 10%-95% of active ingredient, preferably 25%-70%.

The HIV RT may be formulated into neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups) which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups may be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, and ferric hydroxides, and

such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

The HIV RT in adjuvant is administered in a manner compatabile with the dosage formulation, and in such amount as to be immunoeffective either as a therapeutic or preventitive. The quantity to be administered depends on the animal or human to be treated, capacity of the individual's immune system to produce antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgment of the practitioner and are peculiar to each individual.

The above disclosure generally describes the present invention. A more complete understanding can be obtained by reference to the following specific examples which are provided for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

Construction Of A Vaccinia Recombination Vector

According to FIG. 1, the top line represents the genome of HIV-1 and the boxes below the line signify the known genes. RF1, RF2, and RF3 in FIG. 1 indicate the three open reading frames. Each gene is aligned with one of these open reading frames. The *pol* gene consists of a polyprotein encoding protease (prot), reverse transcriptase (RT), and integrase (INT). The RT portion of the *pol* gene was isolated by PCR. The PCR primers shown in FIG. 1 are aligned with the pBH10 sequence. The

PCR fragment produced was digested with Nco I and ligated to pUCK-19. The pUCK-19 vector had been digested with Asp718, treated with Klenow, and digested finally with Nco I. The resulting clone, pUCK-RT, was used to generate the pVV3-RT vaccinia recombination vector.

The remaining part of the example is a detailed description of the construction of the vaccinia recombination vector. A full-length infectious clone of HIV-1, pBH10 (Ratner, et al., *Nature*, 313:277-284, 1985), was used as the source of cloned viral DNA. Oligonucleotide primers were designed such that after PCR, an ATG start codon and a TAG stop codon are at the beginning and end, respectively, of the HIV RT coding region. The oligonucleotide primers and the strategy used in the remainder of the recombinant DNA phase of this project are shown in FIG. 1. The 5' primer was also designed to have a specfic restriction enzyme recognition site (Nco I) at the 5' end to facilitate recombinant DNA manipulations. The amino terminal amino acid of native HIV RT is a proline at nucleotide position 1909. The PCR-derived HIV RT required the addition of methionine and valine at the amino terminal end to allow translational initiation at the Nco I restriction site (see FIG. 1). PCR was performed using the two primers shown in FIG. 1 in a 100μl reaction volume containing containing 10mM Tris-HCl (pH 8.3), 50mM KCl, 1.5mM $MgCl_2$, 0.1% gelatin, 100 pmoles of each primer, and 1 ng of pBH10. The reaction mixture was subjected to thermal cycling as follows: 94°C for 1.0 min.; 45°C for 1.0 min.; and 72°C for 3 min. This cycle was repeated for 30 times and the amplification was terminated with the final 72°C incubation held for 10 minutes.

The 1691 base pair PCR fragment was purified by preparative agarose gel electrophoresis, digested with Nco I, and inserted between the Nco I site and Klenow-treated Asp 718 site of pUCK-19. pUCK-19 is a vector specially engineered for insertion of eukaryotic genes. It was designed to include a consensus eukaryotic ribosome binding site, Kozak sequence GCCACCATGG (Kozak, *J. Mol. Biol.*, 196:947-950, 1987). pUCK-19 was constructed by inserting a synthetic oligonucleotide containing the sequences shown in FIG. 1 between the Bam HI and Kpn I sites of pUC19 using standard procedures (Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982). The validity of the resulting pUCK-RT clone was verified by determination of the DNA sequence at the 5' and 3' ends of the HIV RT gene in this plasmid. The DNA sequence confirmed the presence of the ATG start codon and the TAG stop codon in the proper reading frame. Furthermore, the 200bp of HIV RT coding sequences that were determined at the 5' and 3' ends of the gene matched exactly the published DNA sequence of the pBH10 clone (Ratner, et al., *Nature*, 313:277-284, 1985). The pUCK-RT clone was then digested with Bam HI and Sac I and the HIV RT fragment was inserted into the same sites of pVV3 (see FIG. 1). pVV3 is a vaccinia recombination vector developed by Dr. Denis Hruby at Oregon State University, Corvallis, OR (Thomas, et al., *Science*, 232:1641-1643, 1986).

EXAMPLE 2

Marker Transfer Of pVV3-RT Into Wild-type (wt) VV

Introduction of the HIV RT gene into the VV genome was accomplished by cotransfection of pVV3-RT and wtVV DNA into Ltk$^-$ cells obtained from Dr. Denis Hruby at Oregon State University, Corvallis, OR. Ltk$^-$ cells were seeded onto 100mm culture dishes at approximately 50% confluency on day one. On day two the cells were infected at a multiplicity of infection (MOI) of 0.05 with wtVV. The infection was allowed to proceed for exactly 3 hours before the additon of the DNA. DNA for transfection was prepared using the Lipofectin reagent according to the manufacturer's instructions (Bethesda Research Laboratories). Twenty μg of pVV3-RT plasmid DNA was mixed with one μg of wtVV DNA and used for the cotransfection step. After 24 hours, the infected cells were harvested by scraping the cell monolayer, and a crude VV stock was prepared by three consecutive freeze thaw cycles, which lysed the infected cells. Further amplification of the VV occurred in mouse Ltk$^-$ cells grown in BUdR (25μg/ml) containing medium. These mouse Ltk$^-$ cells were selected for those vaccinia viruses that had lost the wt thymidine kinase (tk) gene as a result of recombination with the tk sequences that flank the HIV RT sequences in pVV3-RT (see FIG. 1). This was accomplished by infecting BudR-selected Ltk$^-$ cells with the crude stock at an MOI of 0.2 and by making a second crude stock by lysing the cells at 72 hours post-infection.

Viruses from the BudR selection were plated and screened for HIV RT gene sequences by plaque hybridization. Briefly, 100mm dishes of confluent BSC40 cells (ATCC,

Rockville, MD) were infected with 500 plaque-forming units of VV, and the infection was allowed to proceed for 36 hours. The addition to the eight mls of culture medium of five mls of 1% neutral red in PBS for three hours at 30°C stained the cells and allowed visualization of the plaques. The monolayers with vaccinia plaques were transfered to 87mm nitrocellulose filters by first draining the medium, then placing the filters directly onto the cells. The filters were lifted from the plates and placed cell side up on PBS-soaked filter paper. A replicate filter was made by placing a second nitrocellulose filter against the first filter. The original filter was stored at 70°C to preserve the viruses in each plaque, while the replica was screened for positive plaques using an approach identical to bacteriophage lambda plaque hybridization (Maniatis, et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 1982). The plaque hybridization procedure used as a probe, ^{32}P-labeled DNA, generated by random-priming DNA from the PCR reaction described above. Several positive plaques were grown and screened for the correct genome structure using restriction mapping procedures of DNA isolated from partially purified VV. Of the several VV:RT recombinant clones resulting from this analysis, VV:RT-3 and VV:RT-24 were selected.

A sample of the vaccinia recombination vector herein designated VV:RT-3 has been deposited with the American Type Culture Collection (ATCC) at 12301 Parklawn Drive, Rockville, MD 20852 USA and has been given ATCC accession number VR 2289.

EXAMPLE 3

Molecular Analysis Of Recombinant Vaccinia Infected Cells

The African green monkey cell line BSC40 was used for all infections. FIG. 2 shows a Western blot of VV:RT infected cell lysates using human anti-HIV sera, Human High Positive Sera (Epitope, Inc.), as the detecting antibody. HIV RT is expressed in the VV:RT infected cells. The Western Blot is performed on protein extracts from vaccinia-infected or mock infected cells. The polyacrylamide gel electrophoresis and electroblotting was performed using standard procedures. Lanes 1-3 are VV:RT isolates #24 and #3, lane 4 is wild-type vaccinia virus (VV:wt); and lane 5 is mock infected BSC40 cells. A 66 kDa band specific to the VV:RT clones is clearly evident. FIGS. 3a and 3b are identical Western blots showing a time course of expression study of RT in VV:RT-infected cells using two different anti-RT monoclonal antibodies (MAb) to detect HIV RT. Lane 1 is a molecular weight marker; lane 2 is mock infected BSC40 cells; lanes 3-5 are pre-infection, T_0; lanes 6-8 are two hours post-infection, T_2; lanes 9-11 are seven hours post-infection, T_7; lanes 12-14 are 24 hours post-infection, T_{24}; and lane 15 is purified HIV. The lane order within each time point is VV:wt, VV:RT3, and VV:RT24. The Western blot in FIG. 3a was developed using 1C11 MAb (Epitope, Inc.) and in FIG. 3b commercially available anti-RT MAb (Cellular Products, Inc.). Both MAbs showed specific and strong reaction with a 66 kDa band that aligns exactly with the native p66 protein from purified HIV (lane 15). It should be noted that VV:RT does not express the HIV protease necessary for processing of HIV RT to the 51 kDa form.

EXAMPLE 4

Purification Of HIV RT For Boosts

HIV RT was purified from VV:RT-infected HeLa cells using a modification of a published protocol (Flexner, et al., *Virology*, 166:339-349, 1988). Briefly, 5 x 10^9 HeLa cells grown in suspension were infected with 5 x 10^{10} pfu of VV:RT-3 and were allowed to grow for 24 hours. The cells were lysed in 25 mls of a hypotonic buffer, and the nuclei pelleted by centrifugation. The supernatant measured 25ml and contained the bulk of the HIV RT. The lysate was adjusted to 75 mM NaCl, 50mM Tris-HCl (pH 8.0), 1mM DTT, and 0.01% NP40 (Buffer A) and applied to a 2.5 x 12.5cm DEAE Sepharose (Pharmacia, Inc.) column equilibrated with Buffer A. Unbound protein was eluted with 100ml of Buffer A. Bound proteins were eluted with a two-step gradient of 150 and 300mM NaCl. Column fractions were analyzed by Western blot for HIV RT using the 1C11 anti-RT mAb (Epitope, Inc.) as the detecting antibody. The bulk of the HIV RT was in the flow through fractions and the 50ml that contained the HIV RT were loaded onto the same sized column containing phosphocellulose (Whatman, Inc.) equilibrated with buffer A. Unbound protein was eluted with 20ml of buffer A, and a 200ml NaCl gradient from 75mM to 300mM was used to elute the HIV RT that had bound to the column. The collected fractions were analyzed for HIV RT on Western blots and for total protein on Coomassie blue-stained polyacrylamide gels. The fractions containing pure RT were pooled and concentrated using a Centriprep 30 concentrator (Amicon, Inc.). The protein concentration of the concentrated preparation was 133 µg/ml, as determined using the BCA Protein Assay Reagent (Pierce).

EXAMPLE 5

Immunization Of Mice And Rabbits

The presence of a yellow, crusty scab at the site of inoculation, approximately five days post-immunization, indicated a successful vaccinia infection in all animals challenged. In addition, all immunized animals demonstrated a positive Western blot response against vaccinia virus proteins. Western blot anaylsis of sera utilizing commercially available HIV-1 viral lysate Western blot strips (EpiBlot™, Epitope, Inc.) indicated that anti-p66 and p51 antibodies were generated in 4/4 vaccinia-RT immunized rabbits as early as 14 days post-inoculation (FIG. 4). The Western blots were developed according to the manufacturer's instructions. Four rabbits (CA, #93, #94, #95) were infected with VV:RT-3 and bleeds were taken at days 0, 7, 14, 35, and 42. These sera were used on the strips at the top of FIG. 4. Hu high+ in FIG. 4 is human high postive anti-HIV sera from Epitope, Inc. In FIG. 4, NRS is normal rabbit sera and anti RT mAb is the 1C11 monoclonal antibody. The bottom half of FIG. 4 shows the antibody response in the same four rabbits following boost with 50μg of purified RT and the entire vaccination regimen in mice. The rabbit sera used are from days 7 and 14 post-boost. Four mice were infected with VV:RT-3 and two control mice with VV:wt. The sera used were from days 14 and 32 post-infection and day 8 post-boost with 50μg of purified RT. NMS in FIG. 4 is normal mouse sera. Sera from all of the VV:RT-infected mice developed a faint p66 band, at 14 days post-inoculation. In contrast, sera from mice immunized with a wiid-type vaccinia preparation and normal mouse sera did

not develop either p66 or p51 bands on the blots (FIG. 4). At 28 days post-inoculation 3 of 4 rabbits were p66 and p51 seropositive. One rabbit designated CA., which was positive at day 14, reverted to a seronegative status at day 42. All four vaccinia-RT mice continued to be seropositive for p66, as well as for p51 at 32 days post-inoculation. The three rabbits seropositive at day 28 continued to test positive against HIV RT (p66, p51) at both 35, 42, and 72 days post-inoculation. The mice and rabbits were boosted with purified HIV RT in Complete Freund's adjuvant and both groups of animals responded by generating high titered antisera against HIV RT. The boosted mice and rabbits were used to generate a panel of anti-RT monoclonal antibodies and high titered antisera, respectively. Sera from two of the boosted rabbits were collected weekly for several months and were analyzed on EpiBlot™ strips as shown in FIG. 5.

EXAMPLE 6

Immunization Of African Green Monkeys

VV:RT-3 was used to induce a humoral and cellular immune response against HIV-RT in African green monkeys (AGM). Each AGM was inoculated by skin scarification with 10^6PFU of vaccinia virus. Six animals were infected with VV:RT and two with VV:wt. As shown in FIG. 6, animals infected with the VV:RT specifically induced antibodies against HIV-RT, as determined by running 0, 3, 4, and 5 week post-infection sera on HIV-1 Western blot strips. In FIG. 6, the top two sets of blots show the anti-RT response in monkeys following infection with VV:wt or VV:RT-3. Each set of 4 strips is from a single animal at days 0, 21, 28, and 35 days post-infection. The bottom panel shows the

boost response to RT in the vaccinated monkeys. Each set of three strips is from a single animal and represents sera from 0, 7, and 17 days post-boost. Animals J261 and J571 were not boosted since they were the VV:wt control animals. The humoral anti-RT response was confirmed by running the sera against Western blot strips generated from either VV:wt or VV:RT infected cell lysates. Although the anti-RT reactivity is weak, a boost with purified RT significantly enhanced this response as was seen in both mice and rabbits.

Four months after infection with VV:RT-3 the boost was administered using 100μg of purified HIV RT in a threonyl-MDP adjuvant (Syntex). Purified HIV RT was dialyzed against PBS (pH 7.2) without Mg^{++} and Ca^{++} and the concentration was determined to be 133μg/ml. The threonyl-MDP was administered at 100μg/kg body weight. The adjuvant was mixed with an equal volume of SAF carrier and administered at 100μg/kg body weight and then injected intramuscularly. Sera were obtained at 7, 17, 21, and 28 days post-boost and analyzed as in FIG. 4 on EpiBlot™ HIV-1 Western blot strips. The results are shown in the bottom panel of FIG. 6. To determine the titer of the anti-RT antibody in the AGM sera, an RT-enzyme-linked immunoabsorbent assay (ELISA) was performed using serial dilutions of the sera from the 28 day bleeds. This assay was performed by coating the wells of a 96-well microtiter plate with 100ng of purified HIV RT and reacting the wells with serial two-fold dilutions of the antisera. RT-specific antibody was detected using peroxidase conjugated to antibody that recognizes AGM antibodies. As seen in FIG. 7, the anti-RT titer of the animals exceeded a 10^6-fold dilution at two weeks post-boost. Using an enzyme-linked immunosorbent assay (ELISA) format, the antibody

titer was measured by determining the dilution the sera could withstand and still have reactivity to purified RT. The titer is represented as the dilution factor of each sera that resulted in a signal two-times background. Each line represents one animal. Animal J261 and J571 were the VV:wt control animals.

The presence of antibodies that inhibit the enzymatic actvity of HIV RT are of clinical relevance. To determine the titer of RT-inhibitory (RTI) antibodies that this vaccine can generate in AGM, an enzymatic assay was developed, and serial dilutions of the AGM antisera were tested for the ability to inhibit HIV RT. As shown in FIG. 8, the titer of RTI antibodies in four of the AGMs exceeded a 12,000 dilution. The assay was conducted using detergent disrupted HIV-1 from actively infected cells and a standard HIV-1 RT assay (Goff, et al., *J. Virol.*, 38:239-248, 1981). This assay was performed using HIV strain IIIb from Hut78-infected cells cultured at Epitope. A standard HIV reverse transcriptase assay (Techniques in HIV Research, Eds. A. Aldovini and B.D. Walker, Stockton Press, 1990, and Goff et al., *J. Virol.*, 38:239-248, 1981) was modified to incorporate a preincubation with monkey sera as follows. Sera were diluted in PBS and 5µl of each dilution was incubated with 5µl of detergent-disrupted cell-free supernatant from the Hut78-infected cultures. The detergent disruption was performed by making the HIV containing culture medium 0.05% NP40. The incubation of virus with sera was performed at 4°C for 30 minutes. The 10µl samples were then added to a 50µl RT reaction mixture and assayed as described in the above cited assays. Data are represented as that dilution at which there is 20% inactivation of the RT activity when compared to negative

control values. The two wild-type vaccinia control monkeys are J261 and J571 and are slhown as having an inhibition titer of 25. Since the lowest dilution measured was a 25-fold dilution and these two sera allowed 100% RT activity at this dilution, their actual inhibition titer is probably much lower.

The cell mediated response of the immunized animals to both HIV RT and VV was assessed by stimulating peripheral blood lymphocytes with the antigen and measuring tritiated thymidine uptake at six days. As shown in Table 1 below, two of the six RT-vaccinated animals had lymphocyte stimulation indices (LSI) that exceeded five. In Table 1, the numbers under the headings of No Antigen and [RT] represent the counts per minute of tritriated thymidine incorporated into cellular DNA. Although only two of six animals demonstrated cellular proliferation in the presence of RT, it is clear that the vaccine described herein is fully capable of eliciting an RT-specific cellular response.

Fresh whole blood from each monkey was used as the source of peripheral blood lymphocytes used in the assay results shown in Table 1. The amount of RT in each well is shown at the top of Table 1. The lymphocytes stimulation was determined by measuring tritriated thymidine uptake at six days. The degree of stimulation is shown as the LSI and is the ratio of the incorporated counts from the antigen-containing wells divided by the counts from the no antigen wells.

Table 1. RT- specific Lymphocyte Proliferation in Vaccinated African Green Monkeys

Animal	No Antigen	[RT]	LSI	[RT]	LSI	[RT]	LSI
		20ug/ml		10ug/ml		5ug/ml	
J261	2031	694	<0	3523	1.7	2221	1.1
J571	19705	17643	<1	18597	<1	20176	1
J574	3016	1787	<1	1998	<1	1697	<1
J903	518	2586	5	3297	6.3	1183	2.3
J904	340	406	1.2	338	<1	156	<1
J907	120	23583	196	32509	270	36673	305
J908	40592	3431	<1	3319	<1	3125	<1
J909	2505	1824	<1	2432	<1	1092	<1

EXAMPLE 7

Construction Of An Adenovirus Recombinant Vector

The starting virus for the adenovirus construct is adenovirus type 5 deletion mutant dl 327 that lacks the Xba fragment from 78.5 map units to 84.7 map units in early region 3 (Cutt, et al., J. Virol., 61:543, 1987). This deletion mutant allows the insertion of DNA without exceeding the amount of DNA that can be included in the virus particle. The removal of the E3 region also prevents production of a virus protein that complexes with the major histocompatibility heavy chain protein and reduces the cell-mediated immune response to the virus. The Bam fragment from 60 map units to the right hand end of the viral DNA is cloned in plasmid. The plasmid DNA is cut downstream of the E3 promoter with a suitable restriction enzyme, and the HIV RT gene inserted in place of the original E3 gene, under the control of the natural

E3 promoter. The resulting plasmid containing the HIV RT gene in the 60 to 100 map unit fragment of dl 327 is cut with the appropriate restriction enzyme to separate viral and plasmid DNA, and transfected into cells together with the overlapping EcoR1 A fragment (0 to 76 map units) of wild type virus. Recombination between the two overlapping DNA fragments will reconstitute viable adenovirus in which the E3 gene is replaced by the HIV RT gene.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made without departing from the spirit or scope of the invention.

CLAIMS

1. A synthetic vaccine, for immunizing an animal or human against HIV, which comprises:

(a) a recombinant vaccine vector, the genome of which comprises, in a non-essential region, a nucleotide sequence capable of being expressed as all or part of an HIV reverse transcriptase; and

(b) all or part of a purified HIV reverse transcriptase in an adjuvant.

2. The synthetic vaccine according to claim 1, wherein the recombinant vaccine vector is a virus.

3. The synthetic vaccine according to claim 2, wherein the virus is selected from the group consisting of a poxvirus, a herpes virus, and an adenovirus.

4. The synthetic vaccine according to claim 3, wherein the poxvirus is vaccinia virus.

5. The synthetic vaccine according to claim 1, wherein the nucleotide sequence is expressed as a 66 kDa subunit of the HIV reverse transcriptase.

6. The synthetic vaccine according to claim 5, wherein the recombinant vaccine vector is a virus.

7. The synthetic vaccine according to claim 6, wherein the virus is selected from the group consisting of a poxvirus, a herpes virus, and an adenovirus.

8. The synthetic vaccine according to claim 7, wherein the poxvirus is vaccinia virus.

9. The synthetic vaccine according to claim 1, wherein the purified HIV reverse transcriptase is a 66 kDa subunit of the HIV reverse transcriptase.

10. The synthetic vaccine according to claim 9, wherein the recombinant vaccine vector is a virus.

11. The synthetic vaccine according to claim 10, wherein the virus is selected from the group consisting of a poxvirus, a herpes virus, and an adenovirus.

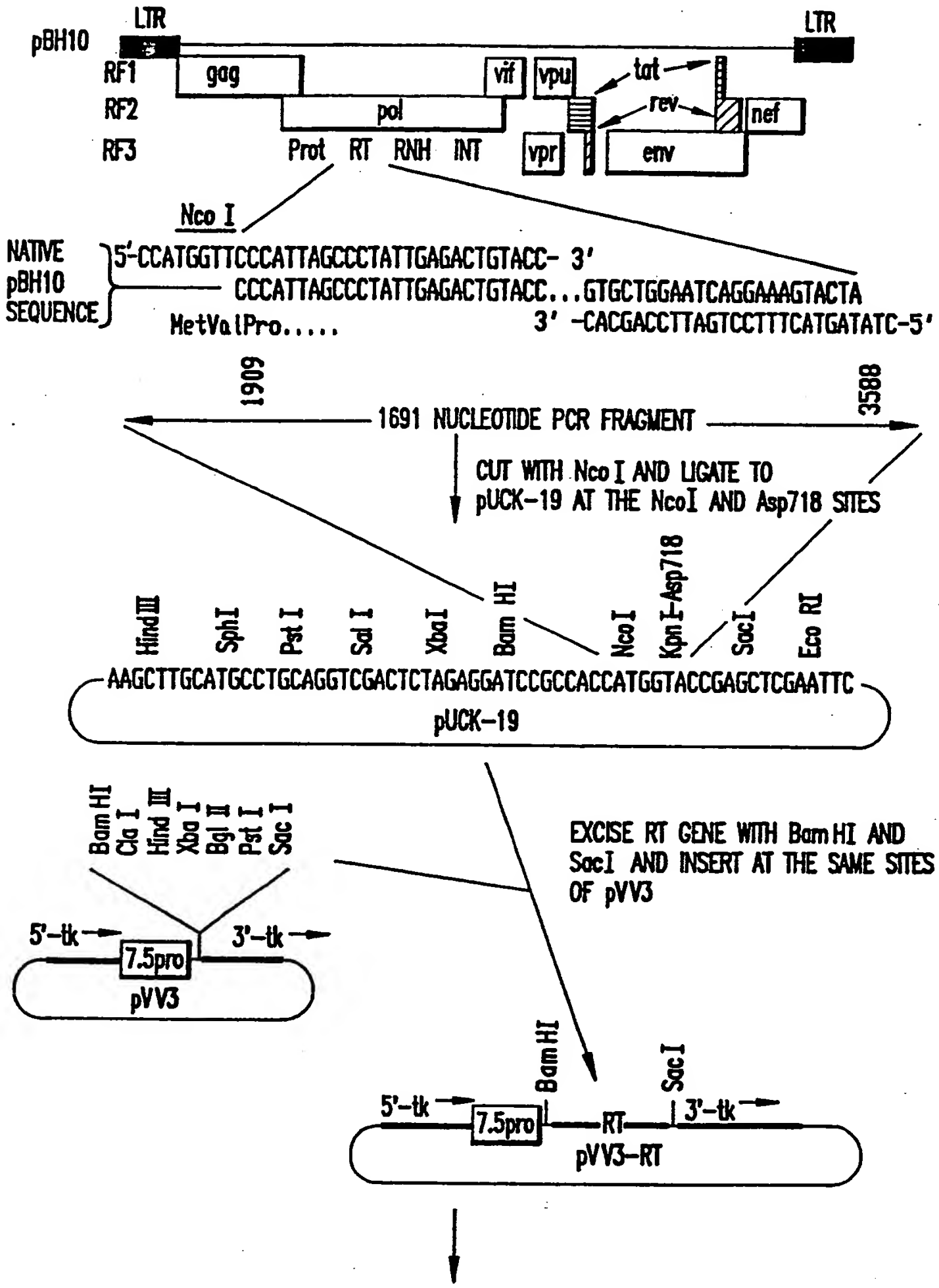
12. The synthetic vaccine according to claim 11, wherein the poxvirus is vaccinia virus.

13. A method for producing an immune response in an animal or human, particularly HIV-infected asymptomatic, immunosuppressed, or immunodeficient animals or humans, which comprises administering to the animal or human a priming inoculation of the recombinant vaccine vector as in any of claims 1-8 or 10-12, followed by a booster inoculation of all or part of the purified HIV reverse transcriptase in the adjuvant, effective in enhancing the immune response of the animal or human to the HIV reverse transcriptase.

14. The method according to claim 13, wherein the purified HIV reverse transcriptase is a 66 kDa subunit of the HIV reverse transcriptase.

15. A synthetic vaccine for use in the treatment of HIV-infected asymptomatic, immunosuppressed, or immunodeficient animals or humans, which comprises administering a priming inoculation of the recombinant vaccine vector as in any of claims 1-8, or 10-12 followed by a booster inoculation of all or part of the purified HIV reverse transcriptase in the adjuvant, effective in enhancing the immune response of the animal or human to the HIV reverse transcriptase.

16. The vaccine according to claim 15, wherein the purified HIV reverse transcriptase is a 66 kDa subunit of the HIV reverse transcriptase.


pBH10
LTR
LTR
RF1
RF2
RF3
gag
pol
vif
vpu
tat
rev
nef
vpr
env
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RT
RNH
INT
Nco I
NATIVE pBH10 SEQUENCE
5'-CCATGGTTCCCATTAGCCCTATTGAGACTGTACC- 3'
CCCATTAGCCCTATTGAGACTGTACC...GTGCTGGAATCAGGAAAGTACTA
MetValPro.....
3' -CACGACCTTAGTCCTTTCATGATATC-5'
1909
3588
1691 NUCLEOTIDE PCR FRAGMENT
CUT WITH NcoI AND LIGATE TO
pUCK-19 AT THE NcoI AND Asp718 SITES
HindIII
SphI
PstI
SalI
XbaI
BamHI
NcoI
KpnI-Asp718
SacI
EcoRI
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCGCCACCATGGTACCGAGCTCGAATTC
pUCK-19
BamHI
ClaI
HindIII
XbaI
BglII
PstI
SacI
5'-tk
7.5pro
3'-tk
pVV3
EXCISE RT GENE WITH BamHI AND
SacI AND INSERT AT THE SAME SITES
OF pVV3
BamHI
SacI
5'-tk
7.5pro
RT
3'-tk
pVV3-RT
GENERATE VACCINIA -RT RECOMBINANTS BY in vivo RECOMBINATION
WITH WILD-TYPE VACCINIA VIRUS

FIG. 1

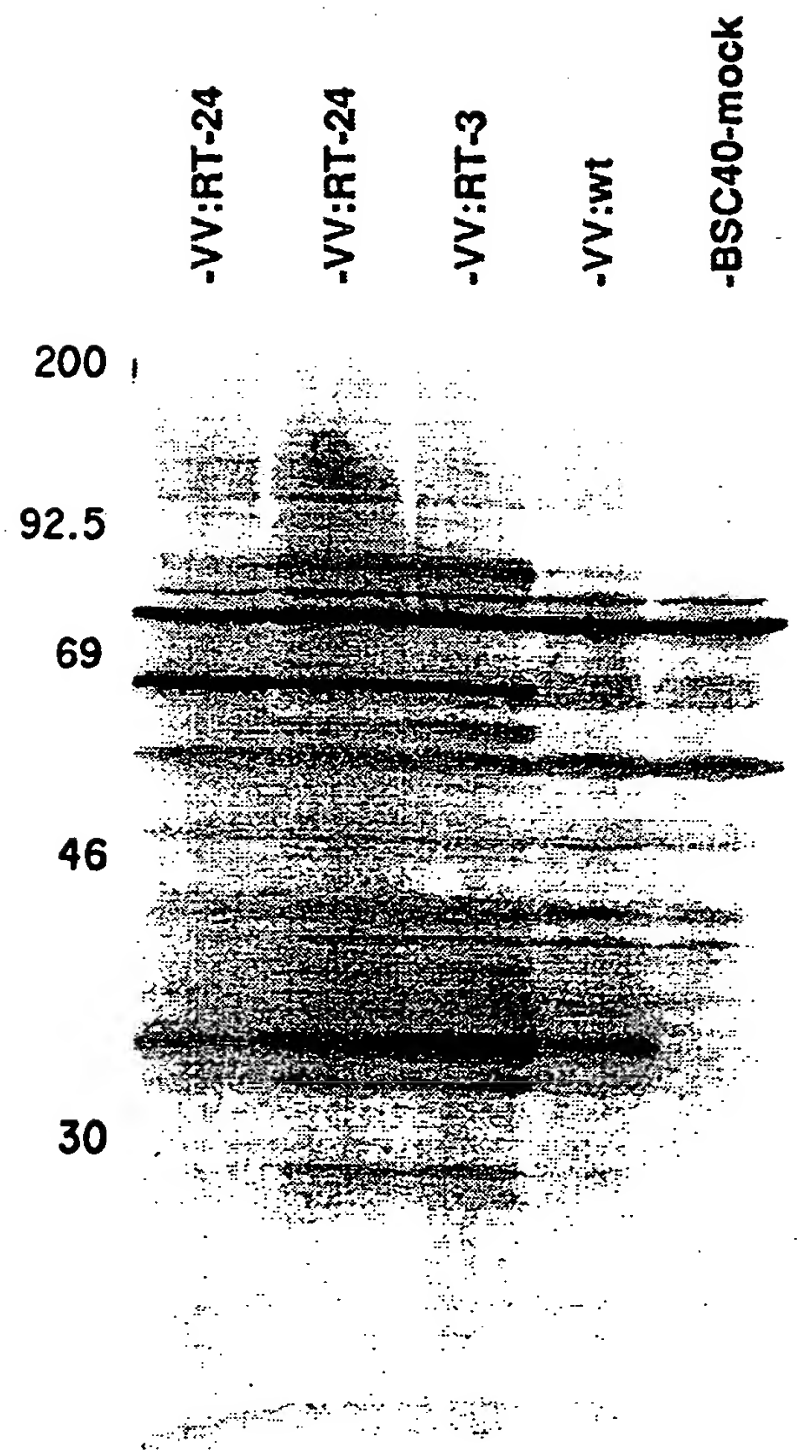
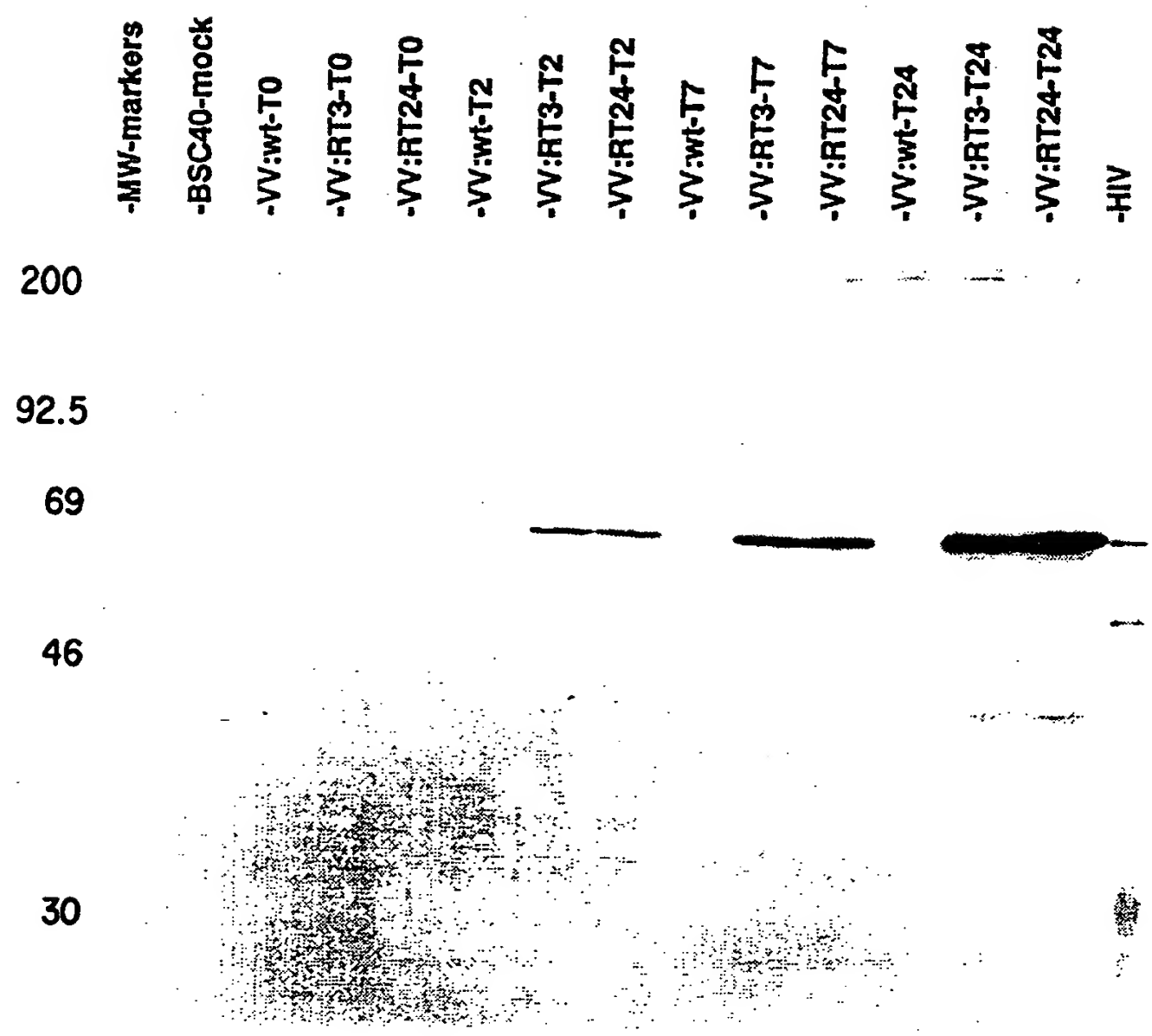


FIG. 2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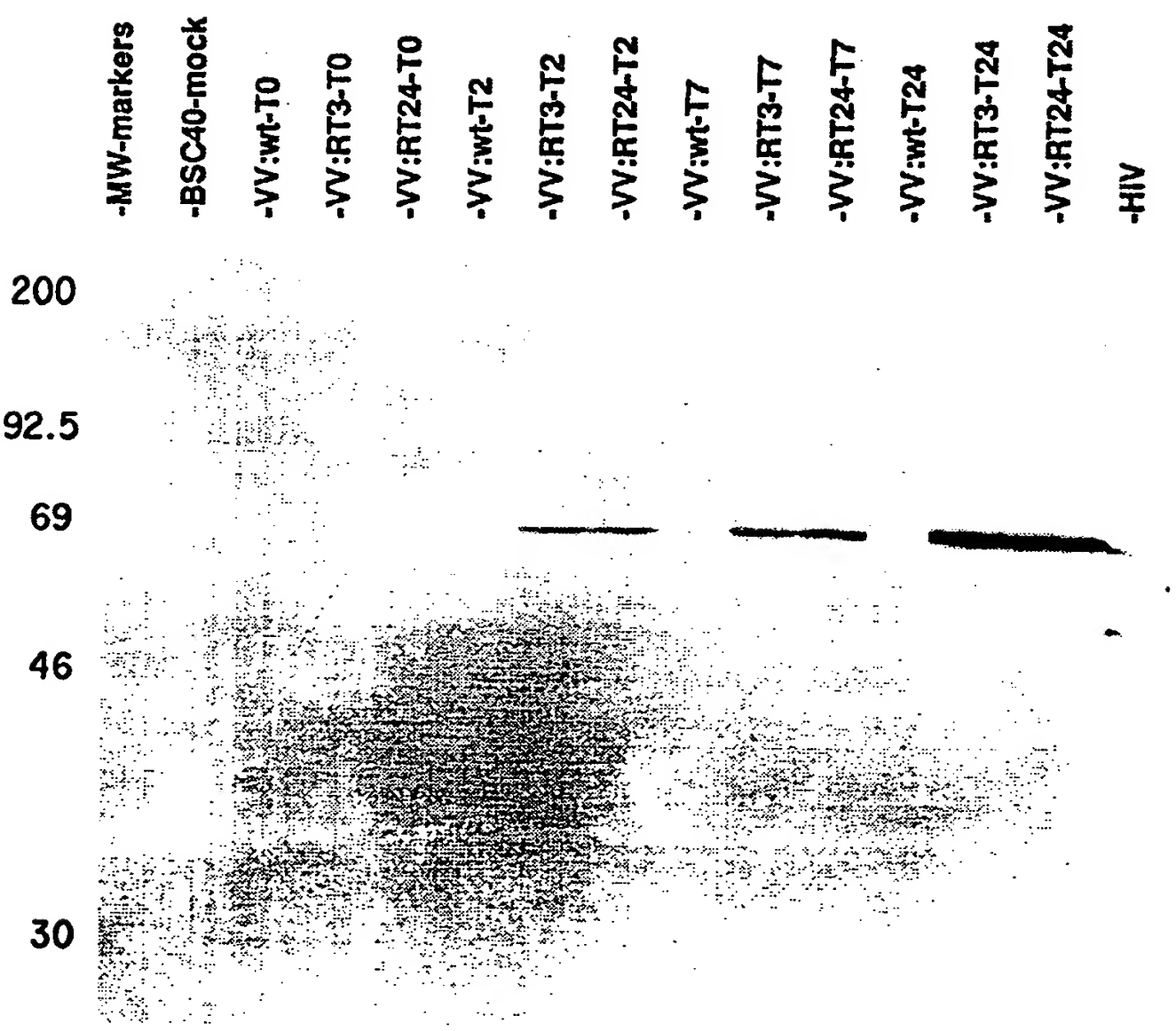
-BSC40-mock
-VV:wt-T0
-VV:RT3-T0
-VV:RT24-T0
-VV:wt-T2
-VV:RT3-T2
-VV:RT24-T2
-VV:wt-T7
-VV:RT3-T7
-VV:RT24-T7
-VV:wt-T24
-VV:RT3-T24
-VV:RT24-T24
-HIV
200
92.5
69
46
30

FIG. 3A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-BSC40-mock
-VV:wt-T0
-VV:RT3-T0
-VV:RT24-T0
-VV:wt-T2
-VV:RT3-T2
-VV:RT24-T2
-VV:wt-T7
-VV:RT3-T7
-VV:RT24-T7
-VV:wt-T24
-VV:RT3-T24
-VV:RT24-T24
-HIV
200
92.5
69
46
30

FIG. 3B

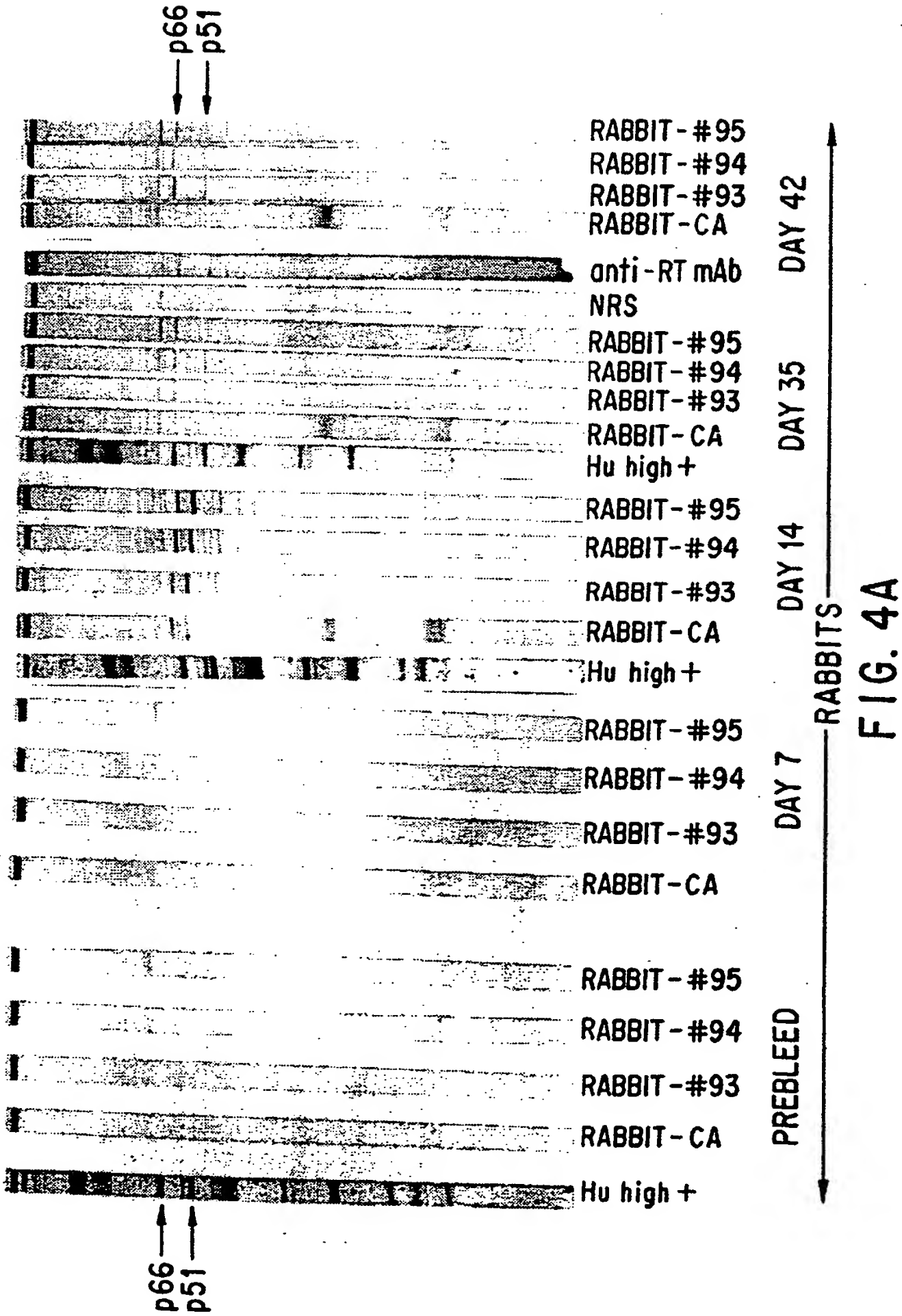
p66
p51
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
anti-RT mAb
NRS
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
Hu high+
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
Hu high+
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
Hu high+
p66
p51
PREBLEED
DAY 7
DAY 14
DAY 35
DAY 42
RABBITS

FIG. 4A

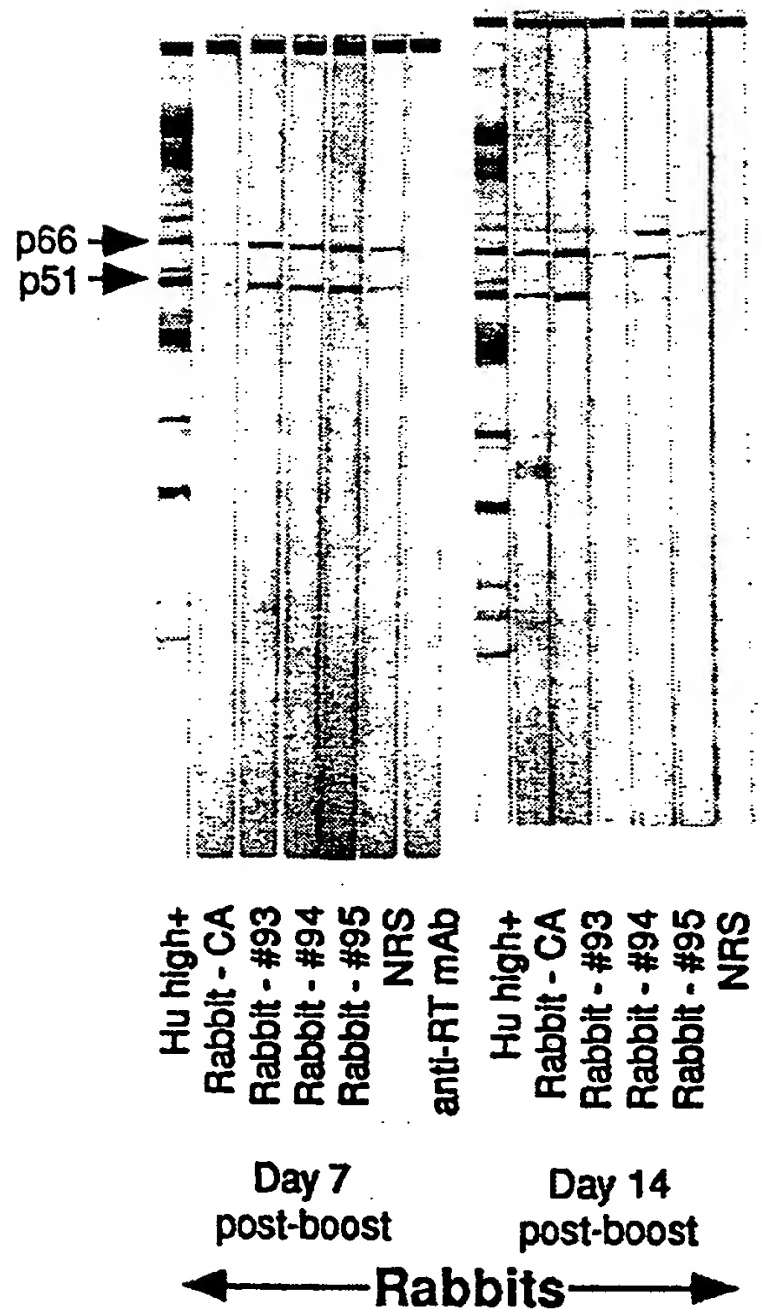
p66
p51
Hu high+
Rabbit - CA
Rabbit - #93
Rabbit - #94
Rabbit - #95
NRS
anti-RT mAb
Hu high+
Rabbit - CA
Rabbit - #93
Rabbit - #94
Rabbit - #95
NRS
Day 7
post-boost
Day 14
post-boost
Rabbits

FIG. 4B

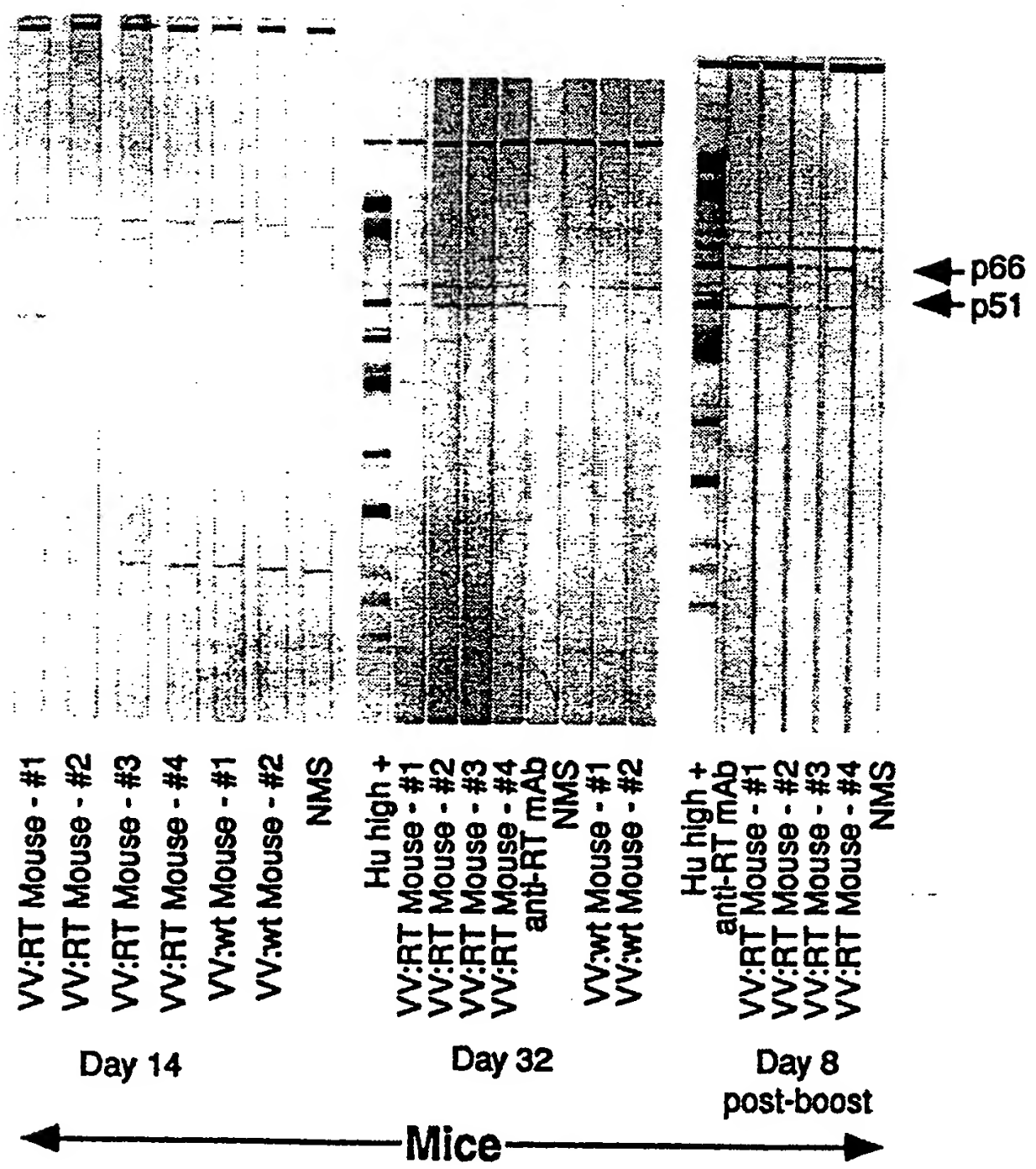
p66
p51
VV:RT Mouse - #1
VV:RT Mouse - #2
VV:RT Mouse - #3
VV:RT Mouse - #4
VV:wt Mouse - #1
VV:wt Mouse - #2
NMS
Hu high +
VV:RT Mouse - #1
VV:RT Mouse - #2
VV:RT Mouse - #3
VV:RT Mouse - #4
anti-RT mAb
NMS
VV:wt Mouse - #1
VV:wt Mouse - #2
Hu high +
anti-RT mAb
VV:RT Mouse - #1
VV:RT Mouse - #2
VV:RT Mouse - #3
VV:RT Mouse - #4
NMS
Day 14
Day 32
Day 8
post-boost
Mice

FIG. 4C

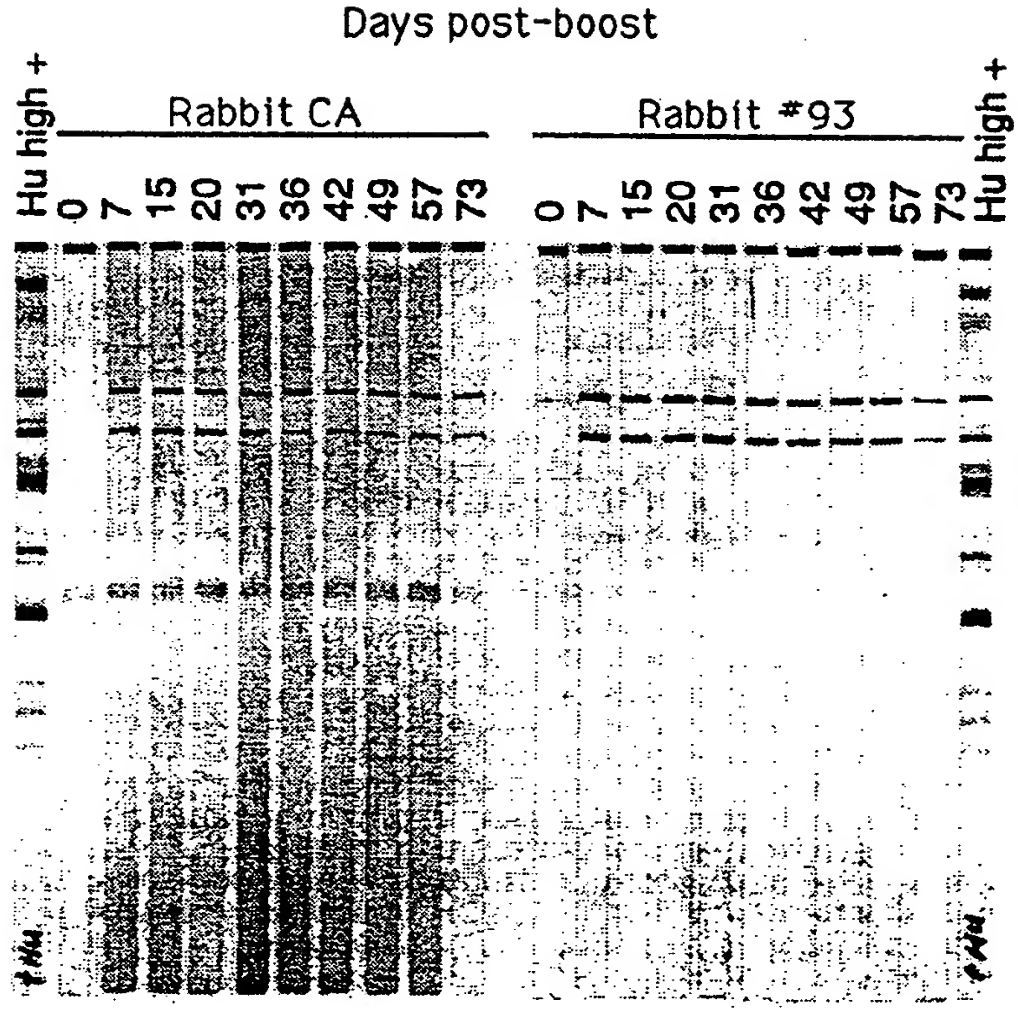
Days post-boost
Hu high +
Rabbit CA
0
7
15
20
31
36
42
49
57
73
Rabbit #93
0
7
15
20
31
36
42
49
57
73
Hu high +

FIG. 5

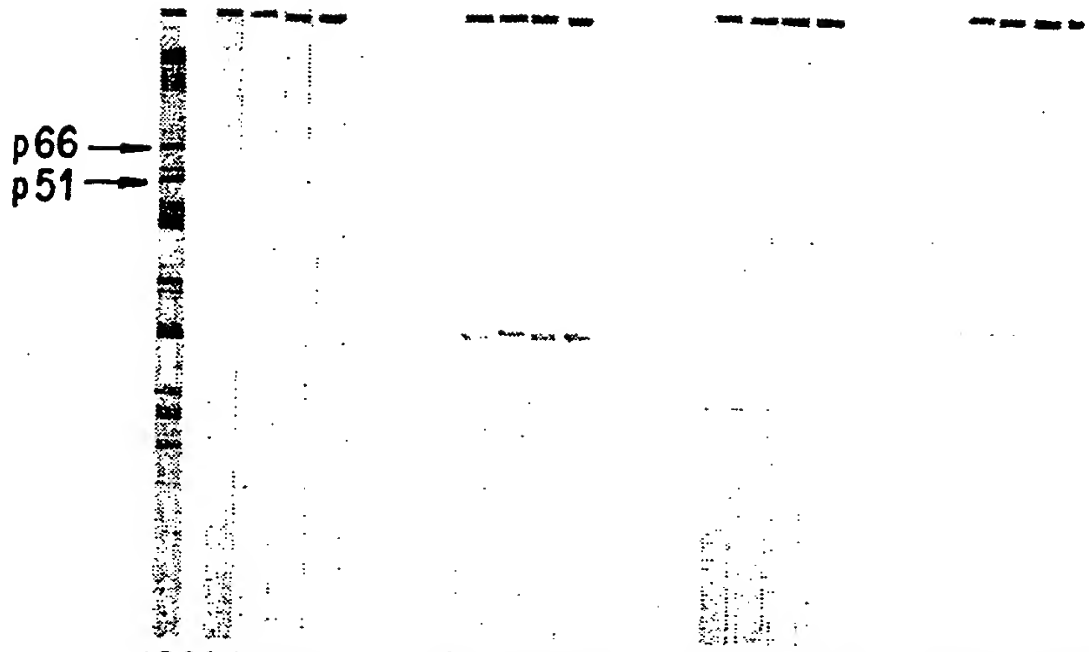

FIG. 6A

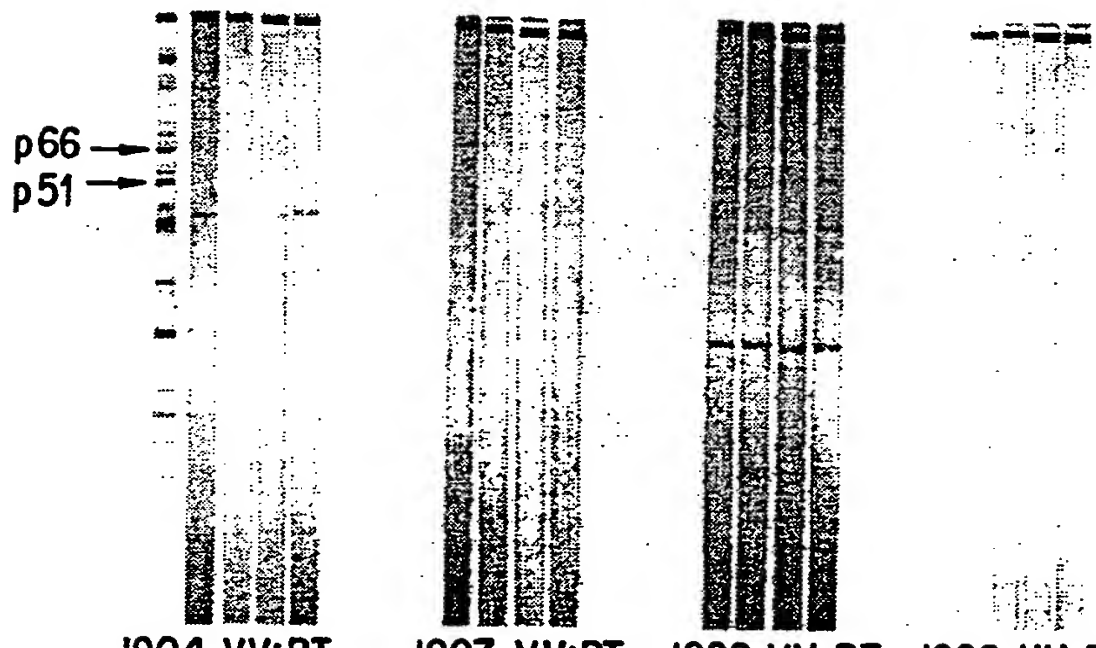

FIG. 6B

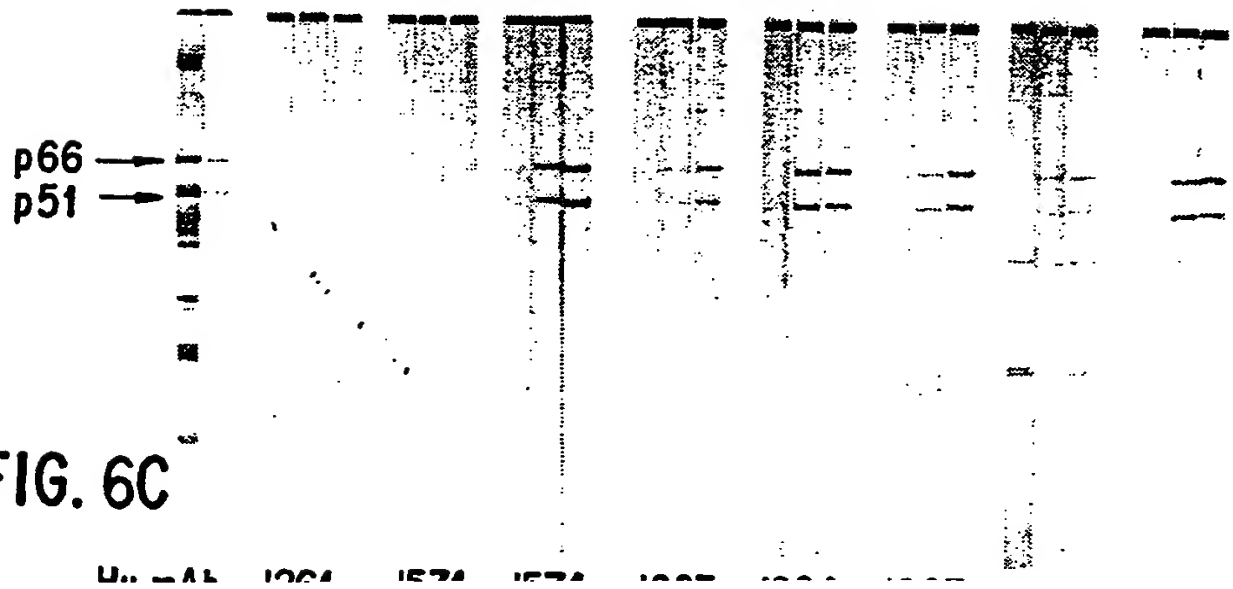

FIG. 6C

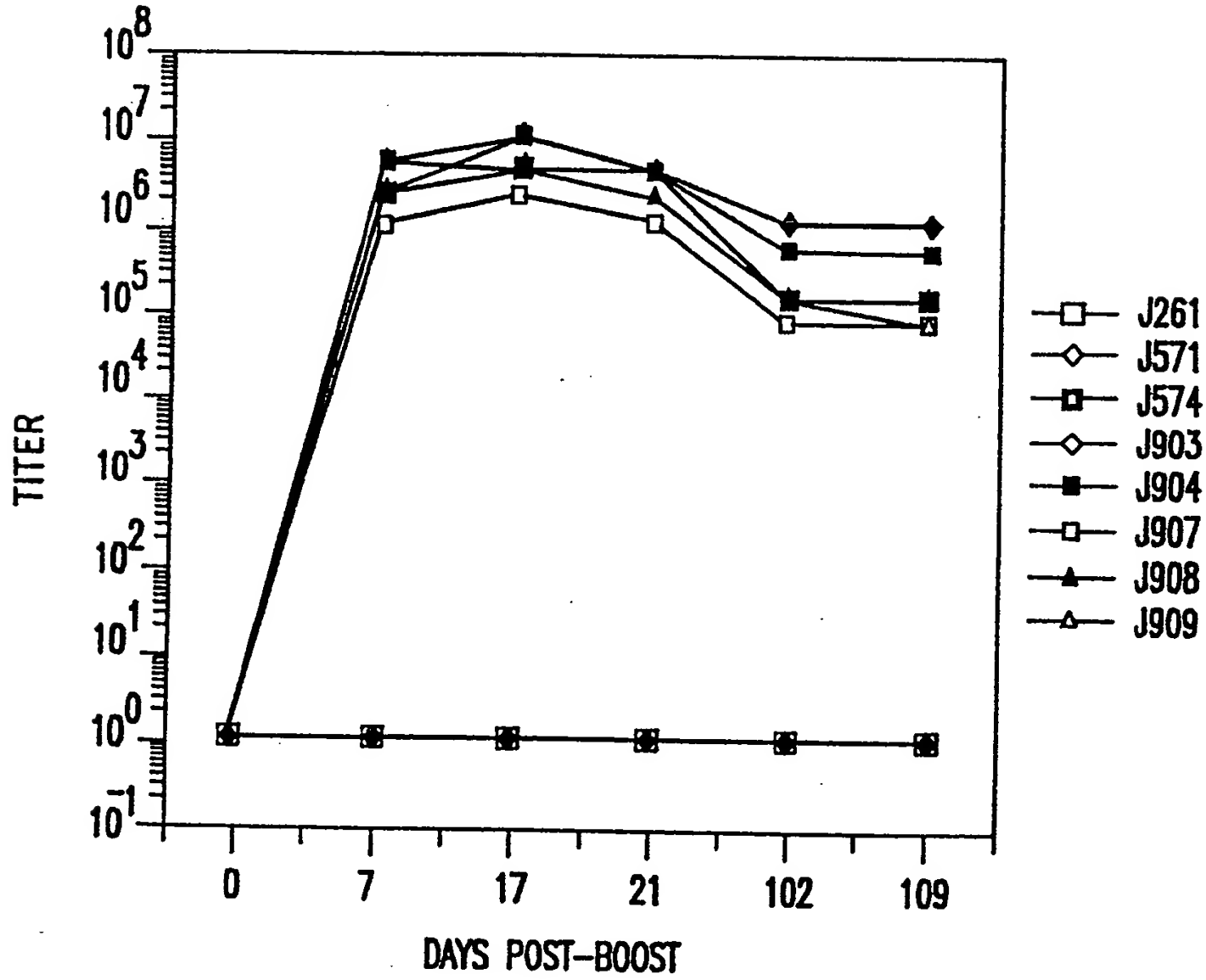
TITER
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
0
7
17
21
102
109
DAYS POST-BOOST
J261
J571
J574
J903
J904
J907
J908
J909

FIG.7

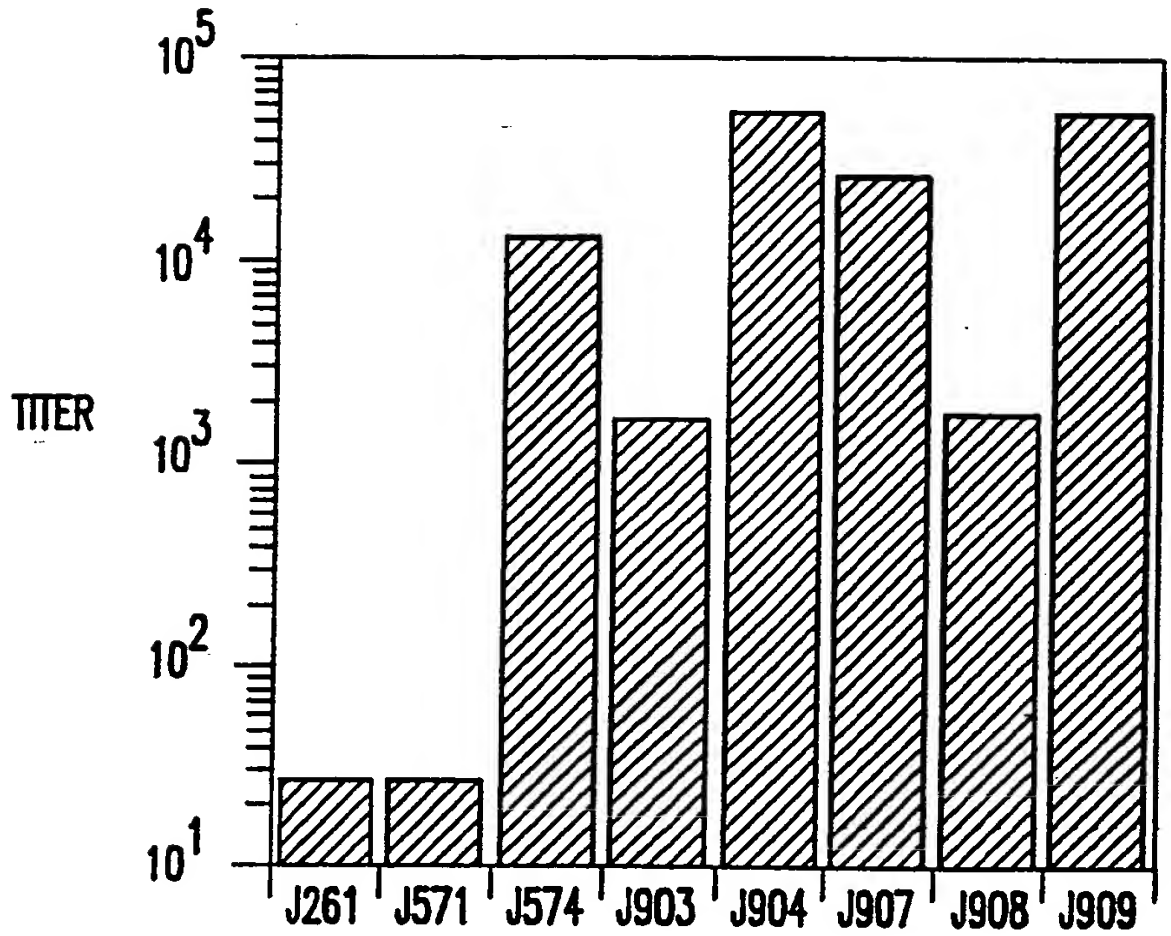
10^5
10^4
10^3
10^2
10^1
TITER
J261
J571
J574
J903
J904
J907
J908
J909
ANIMAL NUMBER

FIG.8

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/09603

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 39/12; C12N 7/00

U.S.CL.: 424/89; 435/235.1

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	424/89; 435/235.1

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

Databases: Dialog(Files Biochem); USPTO Automated Patent System (File USPAT, 1971-1992)

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X/Y	Science, Vol. 236, issued 17 April 1987, Farmerie, et al., "Expression and Processing of the AIDS Virus Reverse Transcriptase in Escherichia coli," pages 305-307, see entire document.	1-12, 15, 16 / 13, 14
X/Y	Science, vol. 240, issued 01 April 1988, Walker, et al., "HIV-1 Reverse Transcriptase is a Target for Cytotoxic T-lymphocytes in infected individuals," pages 64-66, see entire document.	1-12, 15, 16 / 13,14
X/Y	Aids Research and Human Retrovirses, Vol. 4, No. 1, issued 1988, DeVico et al., "High Prevalence of Serum Antibodies to Reverse Transcriptase in HIV-1-INfected Individuals", pages 17-22, see entire document.	1-12, 15, 16 / 13,14
X/Y	Proceedings of the National Academy of Sciences, Vol. 86, issued December 1989, Walker et al.," Long-term culture and Fine Specificity of Human Cytotoxic T-lymphocyte Clones Reactive with Human Immunodeficiency Virus Type 1", pages 9514-9518, See Abstract.	13, 14 / 1-12, 15, 16

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search

25 February 1992

Date of Mailing of this International Search Report

16 MAR 1992

International Searching Authority

ISA/US

Signature of Authorized Officer

Lynette F. Smith gp

Lynette F. Smith

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

X Y	Proceedings of the National Academy of Sciences, Vol. 87, issued March 1990, Hosmalin et al., "An Epitope in Human Immunodeficiency Virus 1 Reverse Transcroptase Recognized by Both Mouse and Human Cytotoxic T lymphocytes", pages 2344-2348, see entire document.	1-4, 9-12, 15 5-8,13,14,16

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

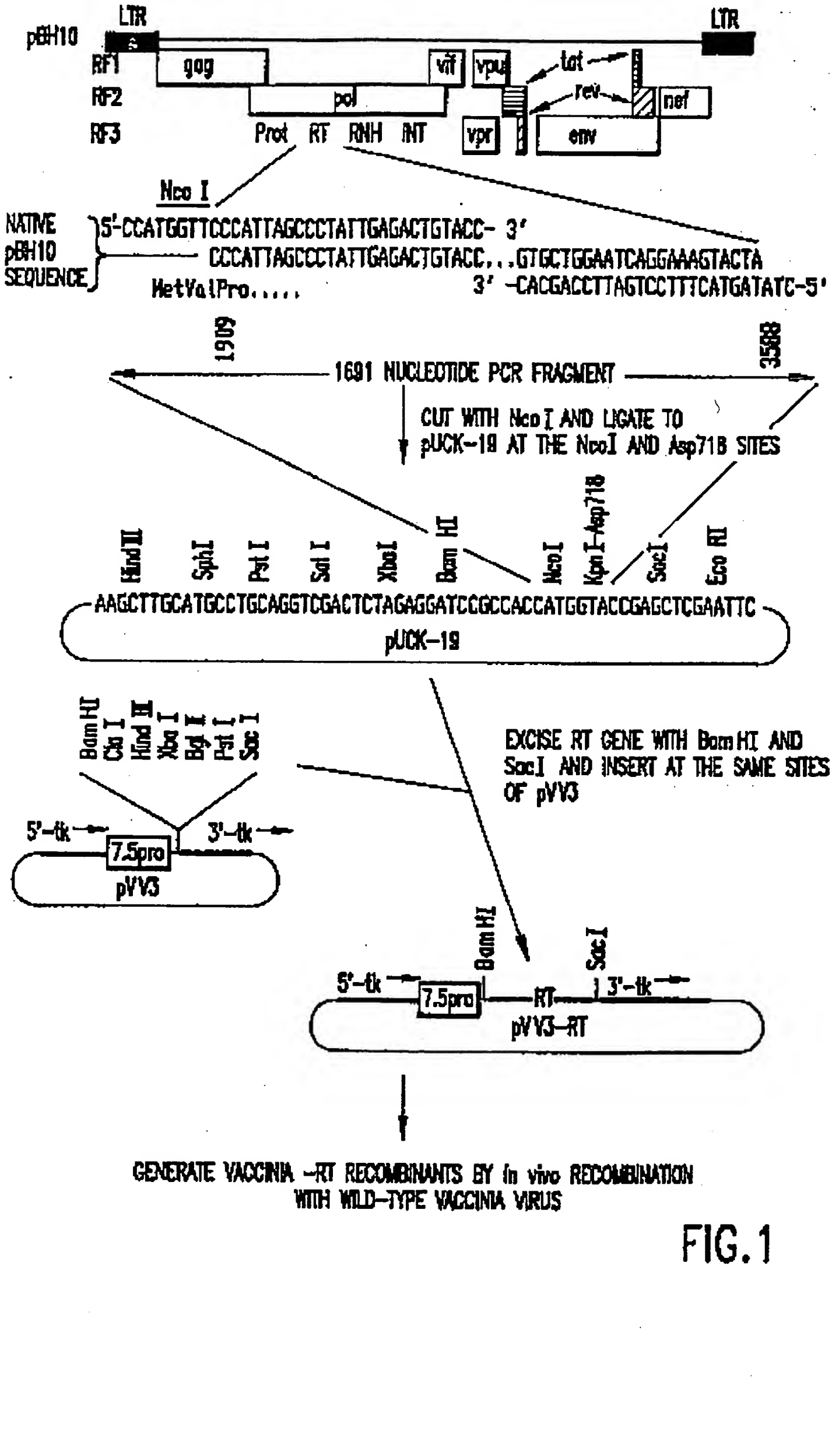
pBH10
LTR
LTR
RF1
RF2
RF3
gag
pol
vif
vpu
tat
rev
nef
vpr
env
Prot
RT
RNH
INT
Nco I
NATIVE pBH10 SEQUENCE
5'-CCATGGTTCCCATTAGCCCTATTGAGACTGTACC- 3'
CCCATTAGCCCTATTGAGACTGTACC...GTGCTGGAATCAGGAAAGTACTA
3' -CACGACCTTAGTCCTTTCATGATATC-5'
MetValPro.....
1909
3588
1691 NUCLEOTIDE PCR FRAGMENT
CUT WITH NcoI AND LIGATE TO
pUCK-18 AT THE NcoI AND Asp718 SITES
HindIII
SphI
PstI
SalI
XbaI
BamHI
NcoI
KpnI-Asp718
SacI
EcoRI
AAGCTTGCATGCCTGCAGGTCGACTCTAGAGGATCCGCCACCATGGTACCGAGCTCGAATTC
pUCK-18
BamHI
ClaI
HindIII
XbaI
BglII
PstI
SacI
5'-tk
7.5pro
3'-tk
pVV3
EXCISE RT GENE WITH BamHI AND
SacI AND INSERT AT THE SAME SITES
OF pVV3
BamHI
SacI
5'-tk
7.5pro
RT
3'-tk
pVV3-RT
GENERATE VACCINIA -RT RECOMBINANTS BY in vivo RECOMBINATION
WITH WILD-TYPE VACCINIA VIRUS

FIG. 1

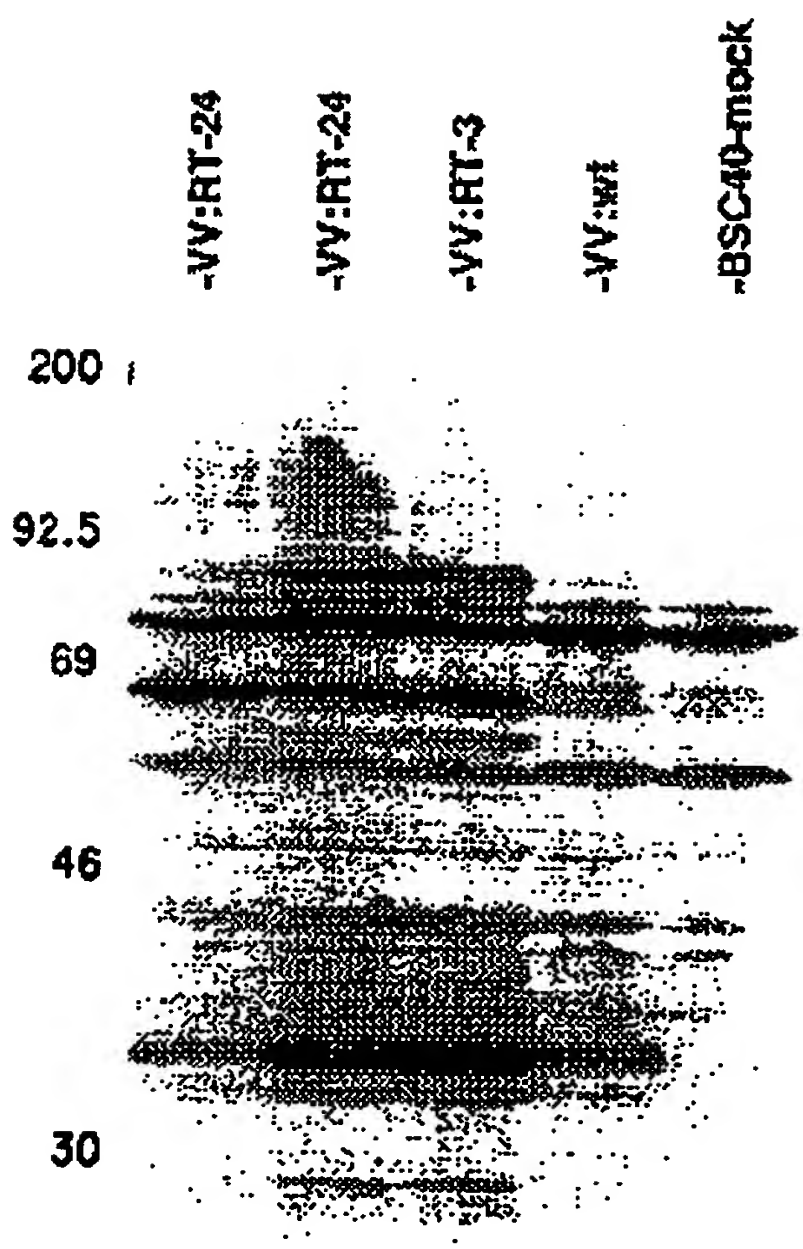

FIG. 2

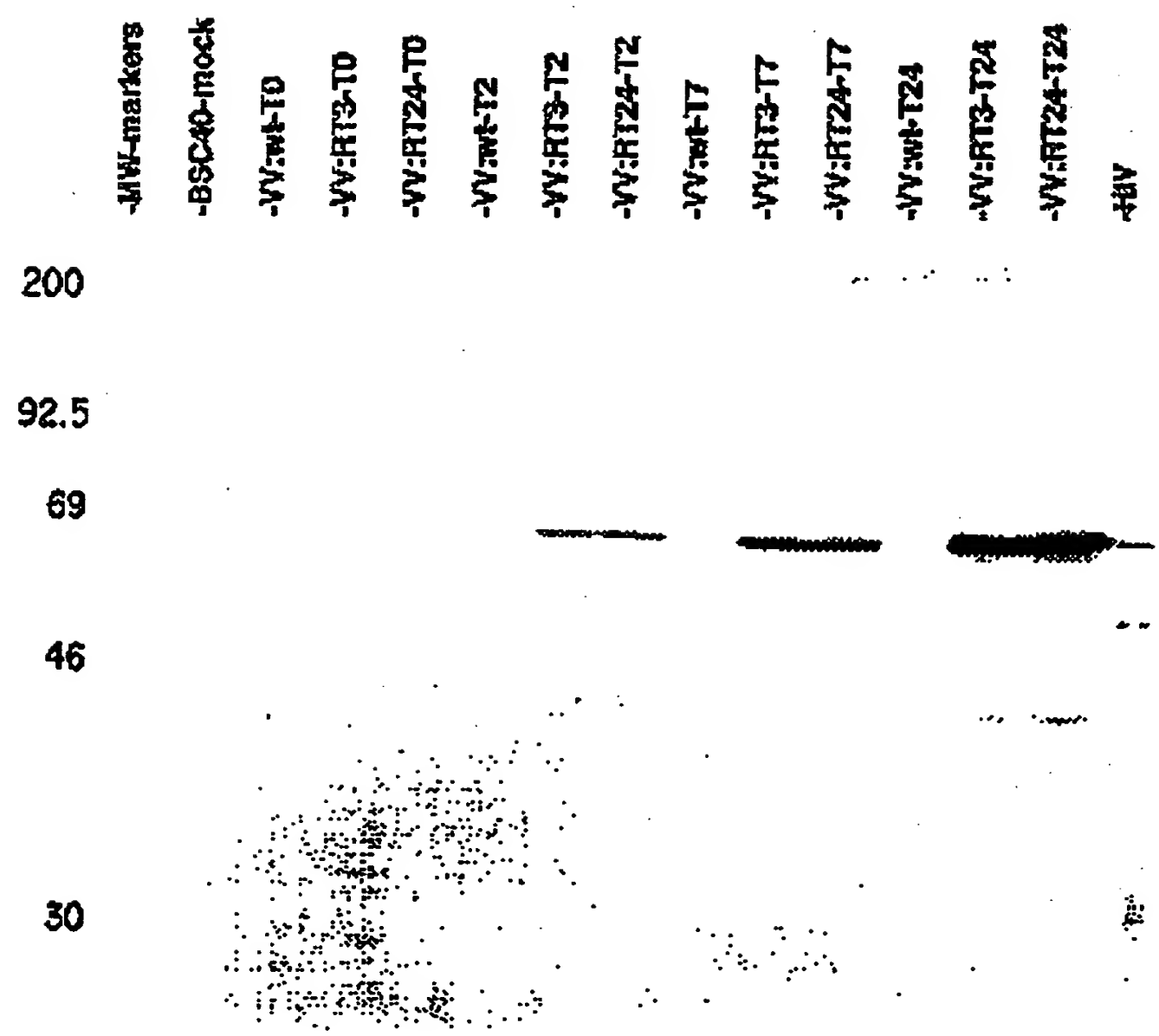
-MW-markers
-BSC40-mock
-VV:wt-T0
-VV:RT3-T0
-VV:RT24-T0
-VV:wt-T2
-VV:RT3-T2
-VV:RT24-T2
-VV:wt-T7
-VV:RT3-T7
-VV:RT24-T7
-VV:wt-T24
-VV:RT3-T24
-VV:RT24-T24
-HIV
200
92.5
69
46
30

FIG. 3A

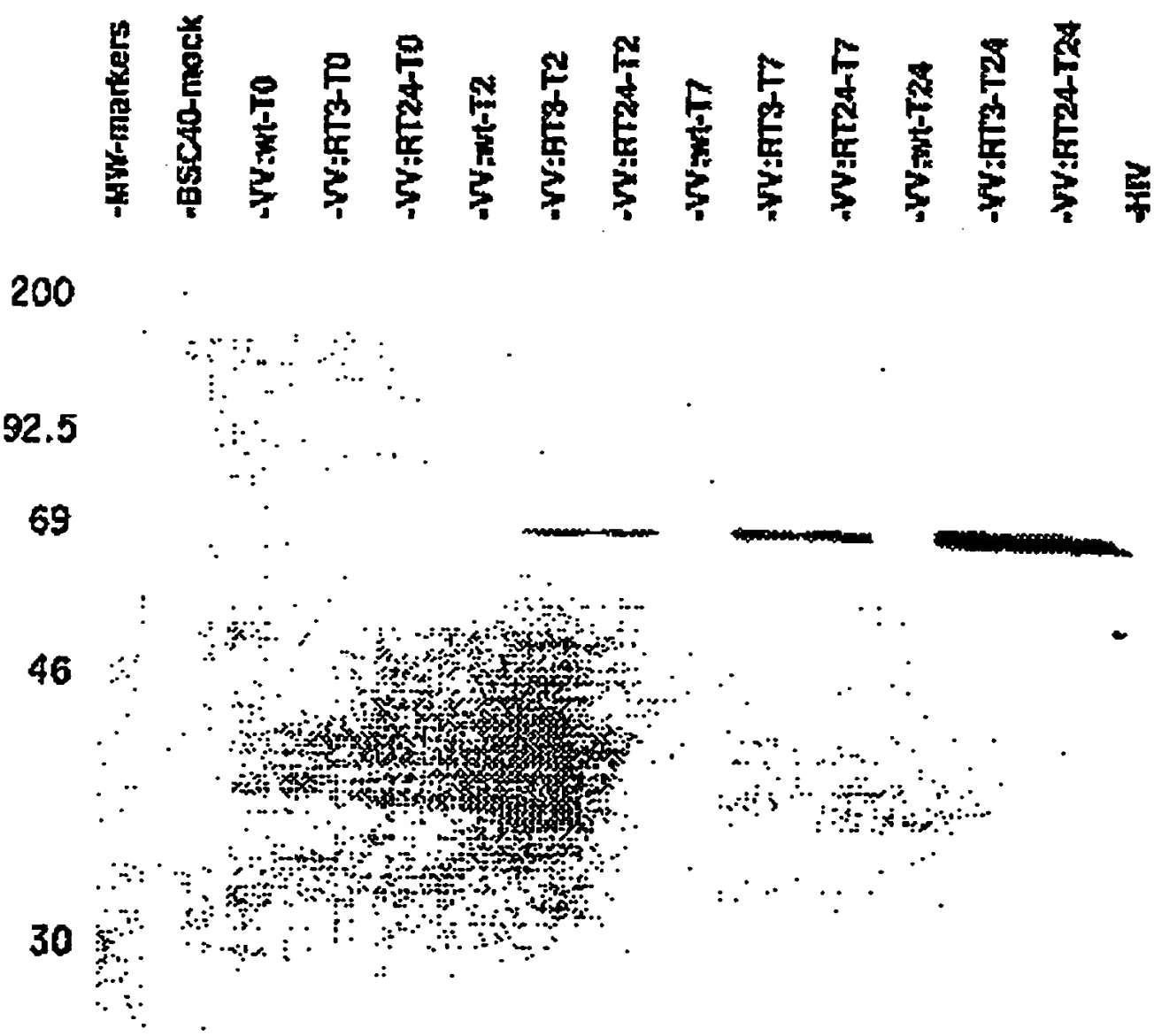
-MW-markers
-BSC40-mock
-VV:wt-T0
-VV:RT3-T0
-VV:RT24-T0
-VV:wt-T2
-VV:RT3-T2
-VV:RT24-T2
-VV:wt-T7
-VV:RT3-T7
-VV:RT24-T7
-VV:wt-T24
-VV:RT3-T24
-VV:RT24-T24
-HIV
200
92.5
69
46
30

FIG. 3B

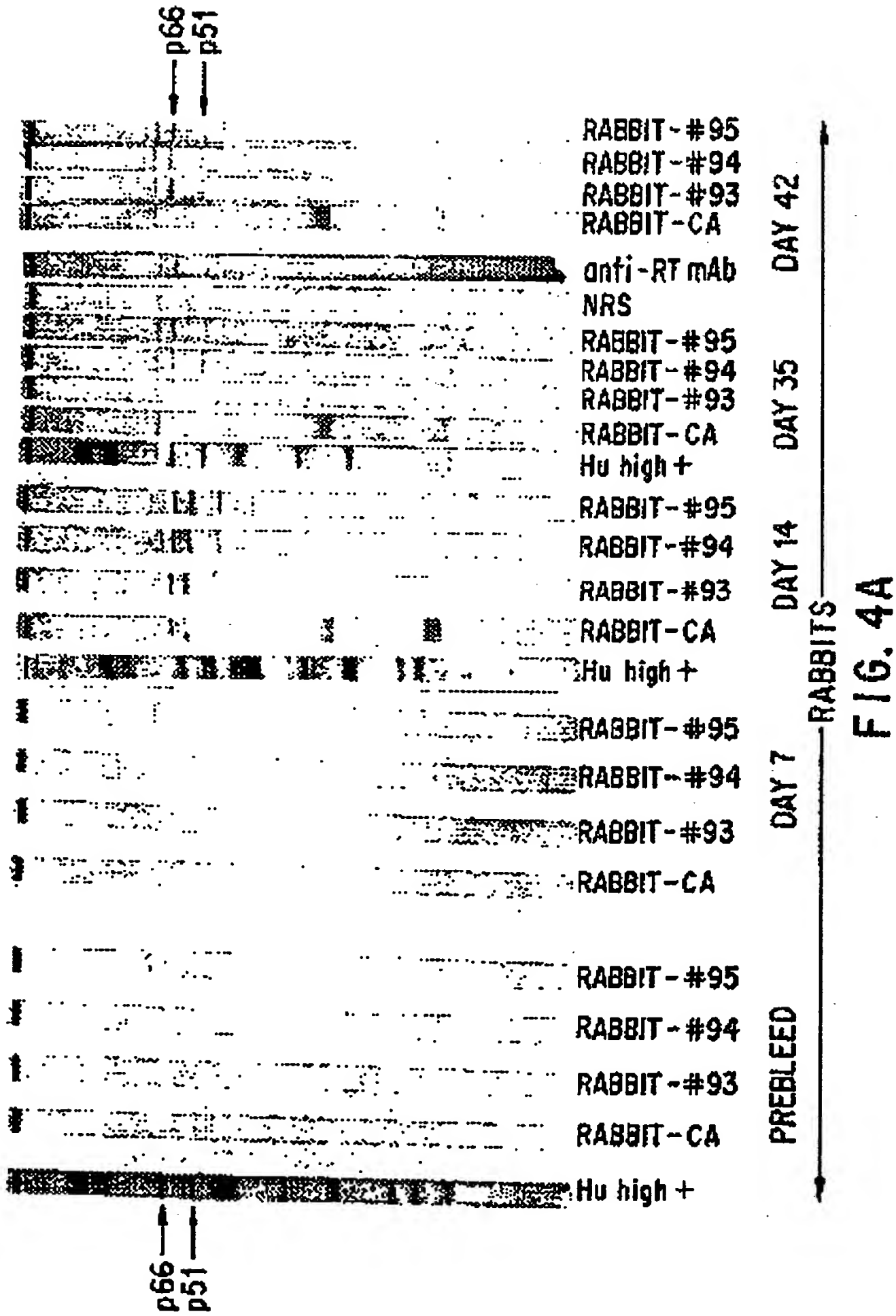
p66
p51
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
anti-RT mAb
NRS
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
Hu high +
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
Hu high +
RABBIT-#95
RABBIT-#94
RABBIT-#93
RABBIT-CA
Hu high +
p66
p51
DAY 42
DAY 35
DAY 14
DAY 7
PREBLEED
RABBITS

FIG. 4A

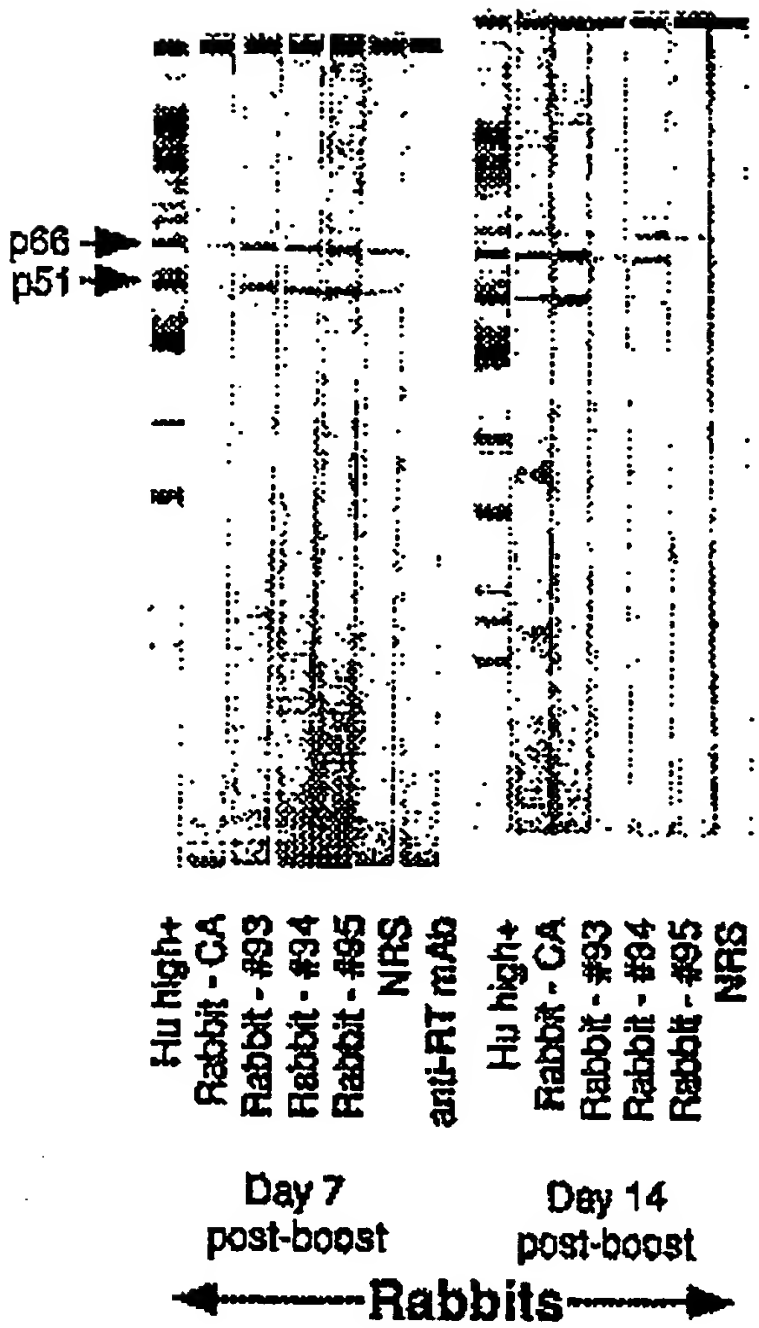
p66
p51
Hu high+
Rabbit - CA
Rabbit - #93
Rabbit - #94
Rabbit - #95
NRS
anti-RT mAb
Hu high+
Rabbit - CA
Rabbit - #93
Rabbit - #94
Rabbit - #95
NRS
Day 7
post-boost
Day 14
post-boost
Rabbits

FIG. 4B

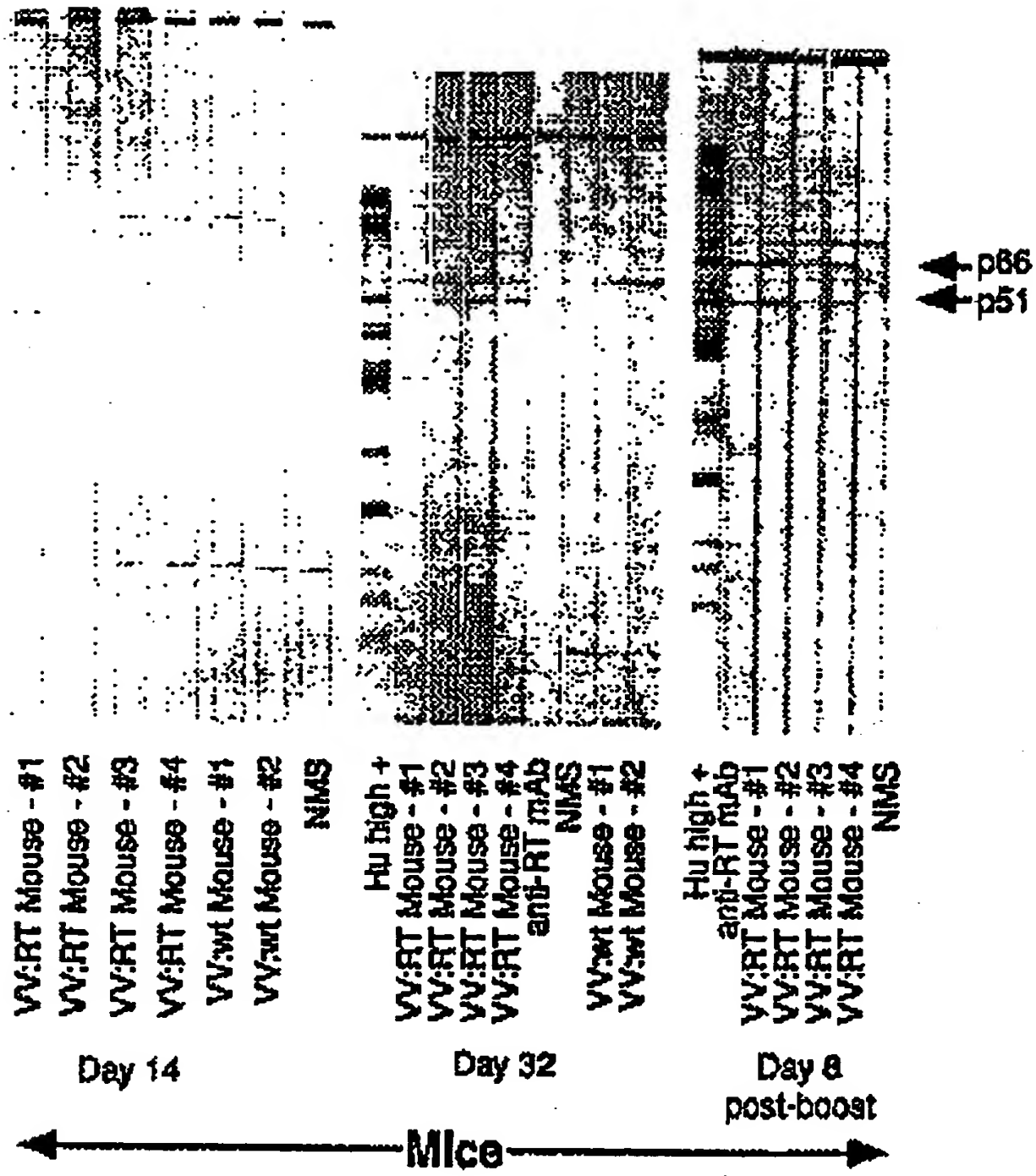
p66
p51
VV:RT Mouse - #1
VV:RT Mouse - #2
VV:RT Mouse - #3
VV:RT Mouse - #4
VV:wt Mouse - #1
VV:wt Mouse - #2
NMS
Hu high + anti-RT mAb
VV:RT Mouse - #1
VV:RT Mouse - #2
VV:RT Mouse - #3
VV:RT Mouse - #4
NMS
VV:wt Mouse - #1
VV:wt Mouse - #2
Hu high + anti-RT mAb
VV:RT Mouse - #1
VV:RT Mouse - #2
VV:RT Mouse - #3
VV:RT Mouse - #4
NMS
Day 14
Day 32
Day 8 post-boost
Mice

FIG. 4C

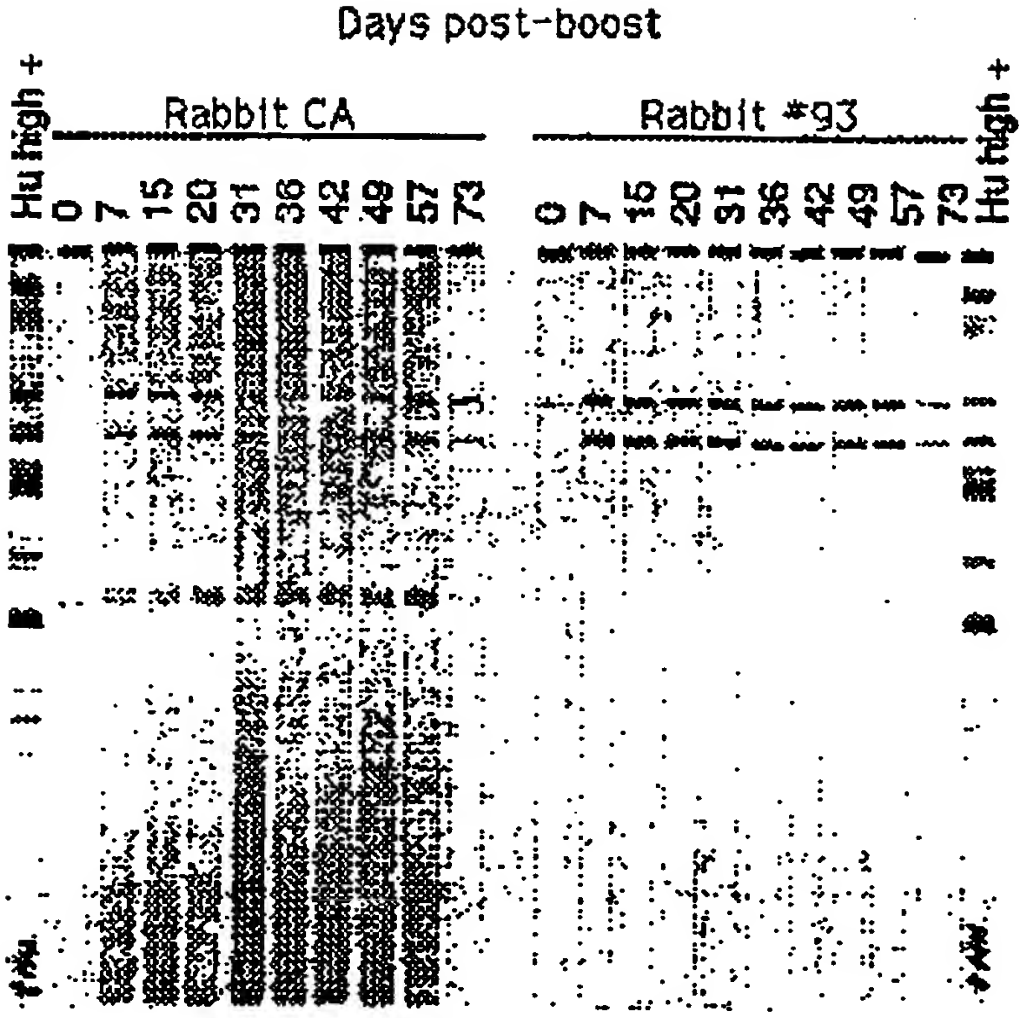
Days post-boost
Hu high +
Rabbit CA
0 7 15 20 31 36 42 49 57 73
Rabbit #93
0 7 15 20 31 36 42 49 57 73
Hu high +

FIG. 5

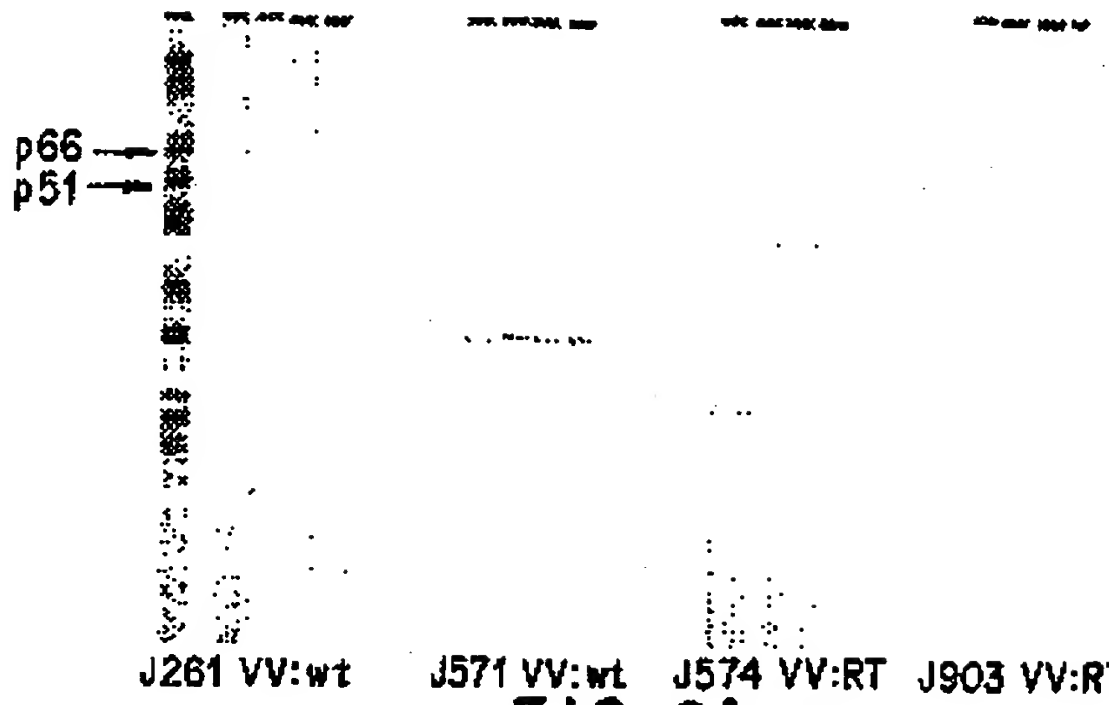

FIG. 6A

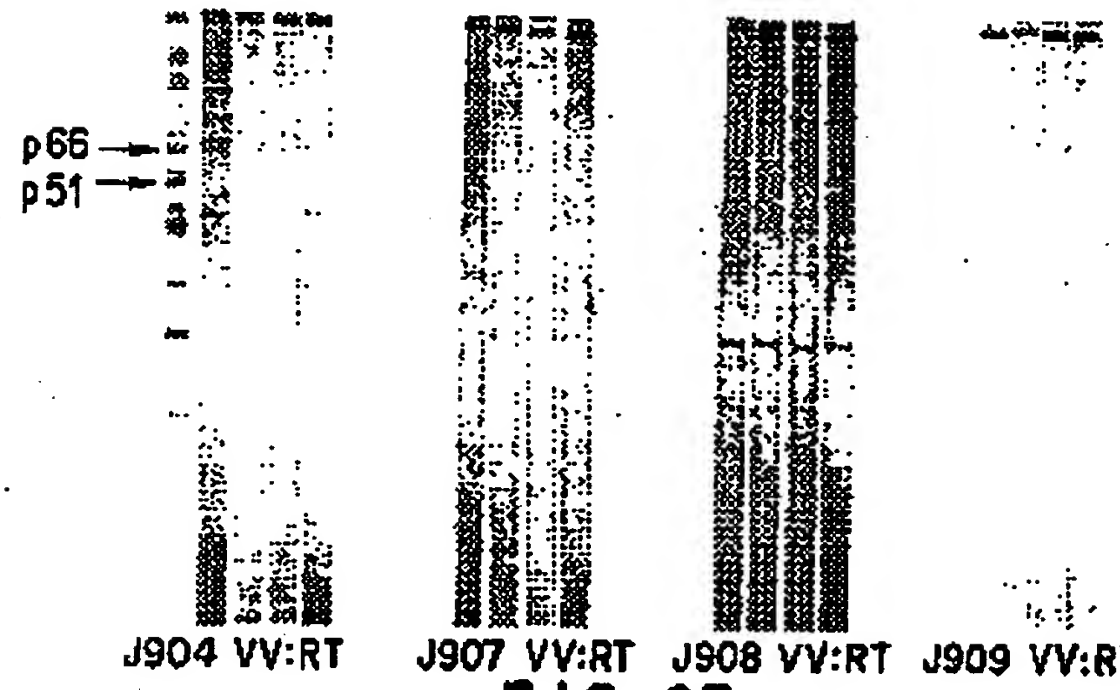

FIG. 6B

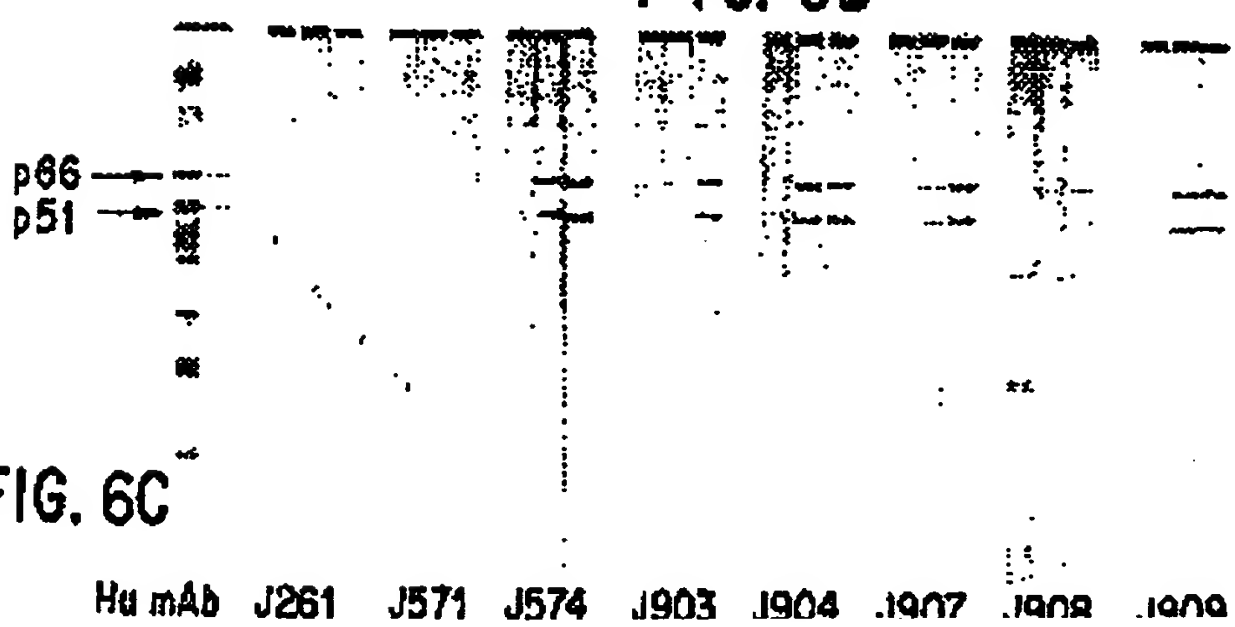

FIG. 6C

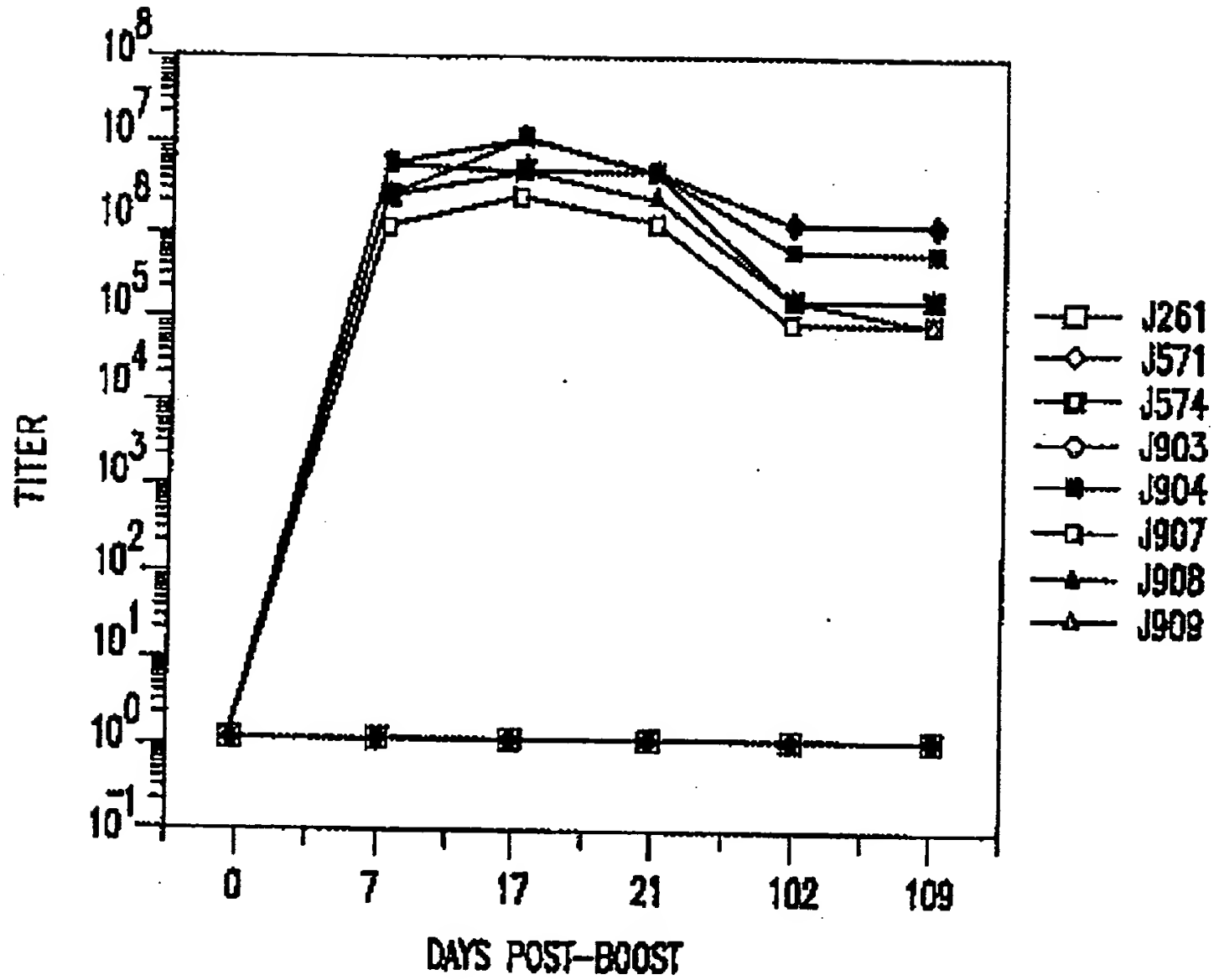
TITER
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
10^{-1}
0
7
17
21
102
109
DAYS POST-BOOST
J261
J571
J574
J903
J904
J907
J908
J909

FIG.7

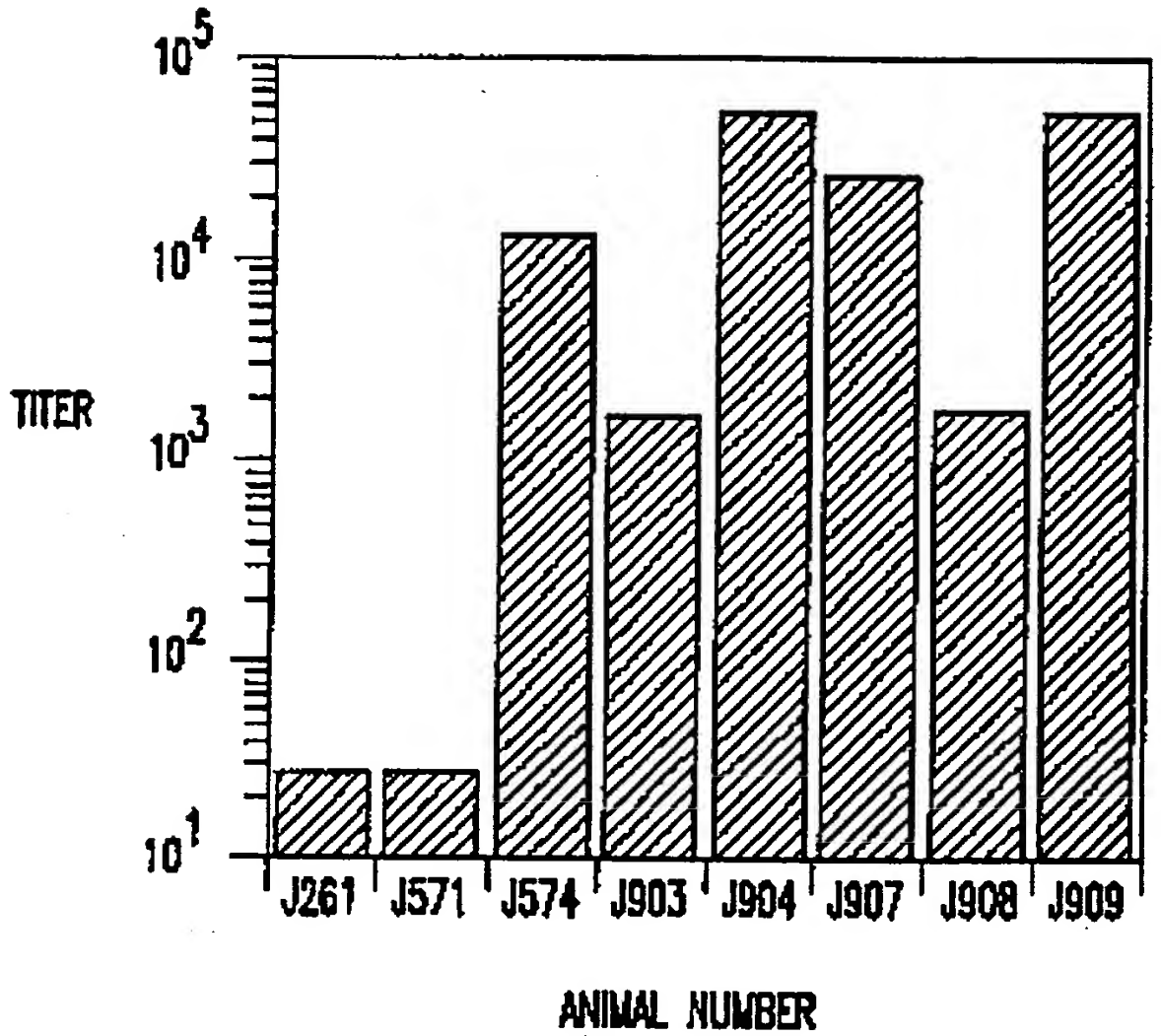
TITER
10^5
10^4
10^3
10^2
10^1
J261
J571
J574
J903
J904
J907
J908
J909
ANIMAL NUMBER

FIG.8